Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Metagenomic insights into kapok-driven nitrogen-cycling hotspots enhancing soil biological resilience in tropical agroforestry systems

SEHRISH ALI
Hainan University
Wen-Qian Xiang
Hainan University
MingXun Ren
Hainan University
WAQAS AHMED
drwaqas@gzhu.edu.cn

Hainan University

Research Article

Keywords: Kapok-rice agroforestry, Bombax ceiba, Nitrogen mineralization, Soil microbial biomass, Extracellular enzymes, Sustainable rice production

Posted Date: November 2nd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10517922/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Intensive rice monoculture in tropical regions is increasingly vulnerable to soil degradation, low nitrogen use efficiency (NUE), and amplified nutrient losses under climate change extremes, necessitating sustainable, biologically driven management strategies. This study evaluated the role of traditional kapok–rice agroforestry, integrating the native red silk-cotton tree (*Bombax ceiba*), in regulating soil nutrient dynamics, nitrogen (N) mineralization, microbial biomass, extracellular enzyme activities, and microbial community composition across five rice growth stages (tillering, booting, heading, maturity, and fallow) compared to rice monoculture systems in Changjiang County, Hainan, China. Using a paired-plot design with three independent replicate fields per system, we compared kapok-integrated soils against adjacent rice monoculture controls. Kapok-integrated soils exhibited markedly enhanced fertility relative to monoculture, with soil organic matter increasing by up to 42%, total N by 44–49%, and available N, P, and K by 63%, 133%, and 93%, respectively, at the maturity stage, compared to monoculture controls. Microbial biomass carbon and nitrogen peaked at 397.17 and 362.40 mg kg^{-1}, accompanied by significantly higher N-acquiring enzyme activities (N-acetylglucosaminidase and leucine aminopeptidase), indicating intensified microbial functioning. Nitrogen mineralization potential (N_o) and cumulative mineralized N were substantially greater (63.42 and 67.46 mg kg^{-1} over 90 days), reflecting improved N retention and cycling capacity under agroforestry management. These enhancements were linked to distinct microbial community shifts dominated by Pseudomonadota, Actinomycetota, Ascomycota, and Bacillariophyta, suggesting increased functional stability and adaptive capacity under climate-sensitive conditions. Overall, kapok–rice agroforestry strengthens soil biological processes, enhances nitrogen cycling, and improves ecosystem resilience, demonstrating its potential as a nature-based, low-input management strategy to mitigate soil degradation and sustain productivity in tropical rice systems under climate change extremes.

1. Introduction

Rice (*Oryza sativa* L.) is the cornerstone of global food security, providing the primary caloric intake for billions of people worldwide (Hoque et al. 2025). To meet the demands of a growing population, modern agriculture has increasingly relied on intensive monoculture systems characterized by high inputs of synthetic fertilizers and pesticides (Young 2012). While this approach has boosted yields, it has come at a significant ecological cost, leading to widespread soil degradation, loss of biodiversity, and compromised sustainability of production systems (Reynolds et al. 2015). Nitrogen (N), a critical macronutrient, is often the most limiting factor for crop growth, yet its application in conventional rice farming is notoriously inefficient. Nitrogen use efficiency (NUE) in rice cultivation often languishes between 20% and 50%, meaning a substantial portion of applied N is lost to the environment (Reynolds et al. 2015; Huang et al. 2022). This surplus N contributes to a cascade of environmental problems, including soil acidification (Guo et al. 2010), eutrophication of water bodies, and the emission of potent greenhouse gases like nitrous oxide (N_2O) (Yule 2010; Abid et al. 2024). These challenges are particularly acute in tropical regions like Hainan Island, China's primary tropical agricultural base.

Decades of intensive farming have led to severe soil degradation, including a 20% decline in soil organic carbon (SOC) and a 0.3-unit drop in soil pH over the last 30 years (Li et al. 2022). The region's hot and humid tropical monsoon climate, with an average annual temperature of 23–25°C and precipitation of 1500–2500 mm, accelerates soil weathering and the decomposition of organic matter, exacerbating nutrient imbalances and soil fatigue (Li et al. 2022; Qi et al. 2023). As Hainan grapples with declining rice self-sufficiency (Wen et al. 2024) and the mounting pressures of climate change, there is an urgent need for sustainable agricultural practices that can restore soil health, enhance nutrient cycling, and ensure long-term food security.

Agroforestry, which deliberately combines trees and shrubs with crop or livestock systems, stands out as an effective nature-based approach to address contemporary agricultural and environmental challenges (Jose 2009). By replicating key features of natural ecosystems, these systems improve soil fertility, enhance nutrient-use efficiency, and boost carbon storage (Niguse et al. 2022; Islam et al. 2024). Trees play a central role in nutrient management: their deep root systems draw nutrients from deeper soil layers and transfer them to the surface through litterfall, enriching topsoil with organic matter and vital elements (Beule et al. 2019; Kim and Isaac 2022). This natural nutrient recycling reduces dependence on synthetic fertilizers and limits nitrogen losses via leaching and runoff, promoting a more sustainable and closed-loop nutrient cycle (Kim and Isaac 2022; Elrys et al. 2023). A notable traditional example is the kapok–rice agroforestry system practiced by the Li ethnic group in western Hainan, where the native red silk-cotton tree (*Bombax ceiba*) is intentionally preserved along the bunds of terraced rice paddies (Wang et al. 2022; Xiang et al. 2023). This long-established practice embodies valuable indigenous ecological knowledge; however, the underlying scientific mechanisms particularly its effects on soil nutrient dynamics and the nitrogen cycle remain insufficiently explored. Early research indicates that *B. ceiba* contributes to better soil quality, notably by raising soil organic matter and available potassium levels (Wang et al. 2022), but a thorough understanding of its broader influence on soil biogeochemical processes is still needed.

Agroforestry systems that integrate trees with rice cultivation substantially increase soil SMBC and SMBN compared to conventional monoculture rice fields (Jose 2009; Islam et al. 2024). This improvement is mainly attributed to enhanced organic matter inputs from tree litter fall, root exudates, and decaying residues, supplying diverse carbon and energy sources for soil microbes (Nair 2012). Additional benefits include higher plant diversity, improved soil structure and moisture retention (Kim and Isaac 2022), and the encouragement of beneficial symbiotic relationships, especially with arbuscular mycorrhizal fungi that significantly elevate microbial biomass (Beule et al. 2019). Collectively, these elements establish a more supportive environment, resulting in a richer, more diverse, and highly active microbial community that promotes efficient nutrient cycling, sustained soil fertility, and resilient ecosystem functioning in agroforestry setups (Elrys et al. 2023). To fully harness the potential of this traditional kapok–rice agroforestry system for sustainable agriculture, it is critical to move beyond surface-level observations and investigate the underlying mechanistic connections among the tree (*Bombax ceiba*), soil, microbial communities, and rice crop. A deeper understanding of these interactions

is vital for refining management practices that improve soil N retention, enhance soil health, and minimize the environmental impact of rice production.

In this study, we examined the influence of *Bombax ceiba* on soil nutrient dynamics particularly N cycling and quantified the relationships between N-acquiring extracellular enzyme activities, microbial biomass, and bacterial/fungal community composition across five rice growth stages (tillering, booting, heading, maturity, and fallow) in kapok–rice agroforestry compared to rice monoculture in western Hainan, China. We tested three hypotheses: (1) soil nutrient pools are elevated in the presence of *B. ceiba* and fluctuate with crop growth stage; (2) temporal and spatial variations in nutrient availability reshape bacterial and fungal diversity and composition; and (3) *B. ceiba* increases N-acquiring enzyme activity and microbial biomass, thereby boosting nitrogen mineralization and plant-available nitrogen. Elucidating these interactions is essential for optimizing nitrogen use efficiency, strengthening soil health, and promoting the long-term sustainability of rice-based agroforestry systems.

2. Materials and methods

2.1 Experimental site description and soil sampling

This research was conducted in Changjiang County on Hainan Island, China (approximately 19° 15'40"N, 108° 60'62"E), a region where kapok (*Bombax ceiba*) rice agroforestry remains widely practiced and well maintained. This area is recognized for humid tropical ferrallitic soils (Wei et al., 2013), monsoon climate, featuring abundant sunshine, an average temperature around 24.7°C and annual rainfall of 1681 mm (Xiang and Ren, 2019). A paired-plot design was adopted, consisting of three independent replicate fields under kapok–rice agroforestry and three adjacent rice monoculture fields (controls), all with similar soil type (ferrallitic), topography, and historical management. Fields were separated by at least 200 m to avoid cross-influence. Within each field, five sampling zones were established along a radial transect from the kapok trunk (at 2, 4, 6, 8, and 10 m) in agroforestry plots, and at equivalent positions in monoculture plots. At each rice growth stage (tillering, booting, heading, maturity, and fallow), three soil cores (0–20 cm depth, 3.8 cm diameter) were collected from each zone, pooled per zone, and then composited per field to form one representative sample per replicate field (n = 3 per treatment per stage). The composite was first manually cleared of visible roots, gravel, stones, and other debris, then sieved (< 2 mm), and finally divided into three subsamples. One of the subsamples was air-dried and further sieved (< 0.25 mm) to determine soil physio-chemical properties. Another subsample was maintained at 4°C for incubation assays and microbial biomass measurements, while third subsample was preserved (at − 80°C) for DNA extraction and metagenomics sequencing.

2.2 Soil chemical analyses and enzyme assays

Soil organic carbon (SOC) was quantified utilizing a wet oxidation procedure (Olsen, S.R., Sommers 1982). Subsequently, soil organic matter (SOM) was calculated by multiplying the SOC by a factor of 1.724. Total nitrogen (TN) and total phosphorus (TP) were measured according to the protocols outlined

by Black et al. (2016) and Murphy and Riley (1962), respectively. Total potassium (TK) was assessed through wet digestion (H_2SO_4, $HClO_4$) methods established by (Parkinson and Allen 1975). Soil available N (AN) was measured according to (Rukun 1999). Soil available phosphorus (AP) was determined through sodium bicarbonate ($NaHCO_3$; 0.5 M) extraction, followed by Mo–Sb spectrophotometry, while available potassium (AK) was measured using an ammonium acetate (NH_4-OAc; 0.5 M) extraction method (Khan et al. 2022). SMBC and SMBN were quantified using the chloroform (fumigation) extraction method (Vance et al. 1987), with conversion factors of 0.45 for SMBC (KEC) and 0.54 for SMBN (KEN) applied as described by (Voroney et al. 2008). The potential activities of two nitrogen-acquiring (extracellular) i-e, enzymes N-acetyl-β-D-glucosaminidase (NAG) and leucine aminopeptidase (LAP) were assayed using a fluorescence microplate approach. Specifically, soil (1 g) was homogenized in one hundred mL of ready 50 mM sodium acetate buffer ($C_2H_3NaO_2$), with the buffer pH adjusted to the average pH of the study soils. Aliquots of 200 µL of the buffer and soil suspension, along with 50 µL of the respective substrate solution, were dispensed into black 96 well plates and dark incubated (25°C) for 4 hours. Fluorescence range was subsequently measured by means of a microplate advanced reader (Biotek Synergy 2, Winooski, USA) with the excitation/emission setting (365 nm & 450 nm), respectively.

2.3 Soil Incubation and N Mineralization Measurement

Soil nitrogen mineralization was evaluated using an incubation–leaching method as outlined by Stanford and Smith (1972). Specifically, 20 g of soil were combined in a 1:1 weight ratio with acid washed quartz sand and placed into polyvinyl chloride (PVC) leaching columns. The columns were kept at a stable temperature of 25°C for a 90-day incubation period. At specified intervals (days 0, 10, 20, 35, 50, 70, and 90), each column was leached with 100 mL of 0.01 M $CaCl_2$ solution, followed by 25 mL of a nitrogen-free prepared solution, and the leachates were subsequently collected. The mineral nitrogen (NO_3^--N & NH_4^+-N) present in the leachates was promptly quantified using the continuous flow analyzer machine (FOSS FIASTAR 5000). The cumulative/aggregate nitrogen mineralization was determined as total mineral nitrogen recovered over the 90-day duration. The potentially mineralizable nitrogen (N□) was estimated by applying a first-order (PFO) kinetic model to mineralization data (Zhang et al., 2017).

$$N_{min} = N_o \left(1 - e^{-kt}\right) \quad (1)$$

where, N_{min}: represents cumulative mineralized N (mg/kg), N□: represents mineralizable potential of N (mg/kg) and k: represents net N mineralization rate constant (NMR (d^{-1}). The first order kinetic model optimization for nitrogen mineralization was done using origin-pro (Systat, Chicago, USA).

2.4 DNA extraction and metagenomics sequencing

Soil samples were processed to extract genomic DNA using the Mag-Bind Soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) following the protocol provided by the manufacturer. Purity and concentrations of the extracted DNA were assessed with NanoDrop2000 and TBS-380, respectively. Quality of extracted DNA was assessed with 1% agarose gel, and then fragmented to an average size of around 350 bp using Covaris M220 (Gene Company Limited, China), for paired-end library construction. Then, a paired-end

library was generated using NEXTFLEX Rapid DNA-Seq (Bioo Scientific, Austin, TX, USA). Adapters, containing the full set of sequencing primer hybridization sites, were ligated to the blunt ends of the fragments. Paired-end sequencing was performed on Illumina NovaSeq (Illumina Inc., San Diego, CA, USA) at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China) using NovaSeq 6000 S4 Reagent Kit v1.5 (300 cycles)'s protocol provided by the manufacturer (www.illumina.com).

2.5 Statistical analysis

To evaluate the effects of different stages and distances on soil physico-chemical properties, microbial biomass, enzymes and microbial diversity a two-way ANOVA was applied, and significant main or interaction effects were further examined through pairwise comparisons using least significant difference (LSD) test at $P < 0.05$. Non-normally distributed or heteroscedastic data underwent log or square-root transformation. Microbial community statistics were assessed by means of Bray–Curtis differences and visualized through principle component analysis (PCA) methods. The microbial taxonomic distributions at phylum level were depicted using Circos diagrams via the circlize package (v0.4.16). Variance partitioning analysis (VPA) was performed in R-software in order to check the proportional contributions of different factors affecting the net N mineralization.

3. Results

3.1 Soil Physicochemical Properties

Soil with kapok showed significantly higher values of all the soil properties compared with that of without kapok (Table 1). At maturity stage, soil AN, AP, AK, TN, TP, TK, and SOM levels in both with kapok and without kapok soils were significantly higher ($P < 0.05$), followed by heading stage. The concentrations of above mentioned soil properties were increased by 63% & 58%, 133% & 43%, 100% & 55%, 44% & 49%, 43% & 44%, 62% & 38% and 42% & 16%, respectively in both with kapok and without kapok soils at maturity stage. For SOM, the kapok–rice system showed a mean increase of 42% across all stages relative to monoculture, with modest seasonal variation (15–20%) attributable to spatial heterogeneity in sampling distance from kapok trees rather than true temporal dynamics. Overall, there was a marked significant difference between both soils at each rice reproductive stage.

Table 1
Soil nutrient contents in soil with kapok and without kapok during five stages of rice field. Values are means ± standard deviations. Uppercase letters indicate the interactive effect of stages × kapok that are significantly ($P < 0.05$) different from each other.

Stages	Kapok	AN (mg kg^{-1})	AP (mg kg^{-1})	AK (mg kg^{-1})	SOM (g kg^{-1})	TN (g kg^{-1})	TP (g kg^{-1})	TK (g kg^{-1})
Tillering	With	147.47 ± 4.68 E	4.52 ± 0.44 DE	220.63 ± 6.54 F	32.50 ± 1.12 D	1.78 ± 0.09 D	0.36 ± 0.01 D	23.26 ± 0.34 D
	Without	115.51 ± 5.74 G	3.7 ± 0.16 F	156.63 ± 4.87 H	23.01 ± 0.33 F	1.26 ± 0.05 F	0.24 ± 0.01 F	16.36 ± 1.15 G
Booting	With	163.89 ± 1.67 D	4.83 ± 0.23 CD	262.64 ± 6.6 C	34.20 ± 1.62 C	2.04 ± 0.19 C	0.38 ± 0.01 C	24.59 ± 0.77 C
	Without	131.23 ± 3.49 F	3.9 ± 0.36 EF	173.93 ± 3.82 G	24.13 ± 0.91 E	1.54 ± 0.05 E	0.25 ± 0.01 F	17.85 ± 1.01 F
Heading	With	231.4 ± 3.83 B	7.31 ± 0.7 B	426.35 ± 9.96 B	36.52 ± 2.55 B	2.38 ± 0.07 B	0.48 ± 0.01 B	34.51 ± 0.74 B
	Without	160.02 ± 4.28 D	4.56 ± 0.31 CDE	231.95 ± 7.56 E	25.63 ± 2.73 D	1.77 ± 0.08 D	0.32 ± 0.02 E	21.33 ± 1.5 E
Maturity	With	241.55 ± 0.6 A	10.57 ± 0.77 A	441.39 ± 4.55 A	38.24 ± 1.14 A	2.57 ± 0.09 A	0.51 ± 0.02 A	37.71 ± 0.63 A
	Without	182.57 ± 4.74 C	5.3 ± 0.52 C	242.81 ± 4.57 D	26.80 ± 0.69 C	1.89 ± 0.03 CD	0.34 ± 0.01 D	22.67 ± 0.84 D
Fallow	With	89.87 ± 4.2 H	3.23 ± 0.06 F	109.46 ± 1.85 I	31.77 ± 0.89 D	0.86 ± 0.02 G	0.16 ± 0 G	13.4 ± 0.45 H
	Without	50.03 ± 4.97 I	2.36 ± 0.2 G	36.88 ± 4.46 J	22.64 ± 0.78 F	0.43 ± 0.04 H	0.08 ± 0.01 H	7.65 ± 1.07 I

3.2 Variations in soil microbial biomass and extracellular enzyme assays under with kapok and without kapok systems

The concentrations of SMBC and SMBN in soils with kapok and without kapok were significantly elevated at the maturity stage, compared with those of tillering stage, measuring 397.17 mg kg^{-1} and 362.40 mg kg^{-1}, respectively (Fig. 1a, b). The lowest values were found at the fallow stage as 171.85 mg kg^{-1} and 146.25 mg kg^{-1}, respectively. At fallow stage these concentrations were decreased by 20% and 26% in both the soils compared with the maturity stage. In addition, across all the rice reproductive stages, the biomass concentrations were consistently higher in soil with kapok, compared with the soil without kapok. In a similar way, the potentials of soil extracellular enzymes, NAG and LAP (N-acquiring enzymes) in soils with kapok, exhibited a significant increase at the maturity stage, with concentrations reaching 88.66 & 25.21 mol h^{-1} g^{-1} and 74.37 & 18.53 mol h^{-1} g^{-1}, respectively (Fig. 1b, c). These

concentrations showed an increase of 11.55 & 33.58% and 25.84 & 48.18% compared to the tillering stage. The booting and heading stages had also shown significant increase relative to the tillering stage. Conversely, during the fallow stage, concentrations decreased by 10.75 & 36.18% and 4.81 & 31.15% compared to the tillering stage. Moreover, the soils with kapok tree significantly influenced NAG and LAP concentrations across all stages, compared with those of soils without kapok.

3.3 Temporal variations in soil NH_4^+-N and NO_3^--N contents during incubation under with kapok and without kapok systems

Different rice growth stages significantly influenced soil N content and net N mineralization in both with kapok and without kapok soils (Fig. 2). Over the 90-day incubation period, this significant effect was reflected in the varying concentrations of NO_3^--N & NH_4^+-N. All through the incubation duration, compared to the tillering stage, the booting, heading and maturity stages showed an increased NO_3^--N & NH_4^+-N content by 11.18 & 25.74% and 1.17 & 1.21%, 33.69 & 87.12% and 20.41 & 1.11%, and 46.53 & 103.96%, and 27.66 & 1.21%, respectively. While it decreased at the fallow stage by 7.7 & 5.9% and 8.1 & 46.6%, respectively, compared with the tillering stage in both with and without kapok soils. As compared with the soil with kapok, the without kapok soil had decreased NO_3^--N & NH_4^+-N content at all the stages. At the maturity stage, the concentrations of NO_3^--N and NH_4^+-N were higher; however, they were significantly lower at fallow stage, respectively. Since, the levels of NH_4^+-N and NO_3^--N were higher at all the stages in soils with kapok, the total N concentrations were also higher, lasting till 45 days of incubation period (Fig. 2a-f), however the concentrations remained relatively stable thereafter. Although the nitrogen mineralization dynamics in soils with kapok showed a similar pattern to those of without kapok, they diverged in the later stages during incubation period. Particularly, NH_4^+-N content remained stable at even after 50 days, indicating that the mineral nitrogen released was more readily retained as NH_4^+-N in the soil across different rice growth stages.

3.4 Nitrogen mineralization potentials and mineralization rate constants (k)

Total nitrogen mineralization, distinct as the totality of NO_3^--N and NH_4^+-N, exhibited significant changes throughout the incubation experiment. N mineralization potential (N_o) ranging from 63.42 & 52.65 mg kg^{-1} at maturity stage to 41.26 & 38.26 mg kg^{-1} at fallow stage and nitrogen mineralization rate (k) extended between 0.29 & 0.26 to 0.11 & 0.12, respectively in the both the soils with kapok and without kapok (Table 2). Net nitrogen mineralization displayed a significant curved relationship over the course of the incubation period at different rice growth stages. Upon accomplishment of incubation period, the maximum nitrogen mineralization occurred at maturity stage (67.46 & 55.81 mg kg^{-1}), followed by the heading stage (61.56 & 52.71 mg kg^{-1}), booting stage (50.89 & 43.91 mg kg^{-1}), tillering stage (45.64 & 43.42 mg kg^{-1}) and fallow stage (42.14 & 39.97 mg kg^{-1}), respectively. The total mineralized N in soils with kapok at all the stages was higher, compared with that of soils without kapok. With higher

concentrations of the total mineralized N at maturity stage, the concentrations were also significantly higher in soil by 47.81%, and 27.11% compared with that at tillering stage. Furthermore, the nitrogen mineralization data points showed a strong fit (R^2 = 0.96–0.99) when modeled using the (pseudo) first order (PFO) model. N mineralization rate constant (k) was steady with the different rice growth stages and showed an overall increasing trend starting from maturity to fallow stages.

Table 2
First-order kinetic fitting model parameters for nitrogen mineralization in soil with kapok and without kapok during five stages of rice field. Values are means ± standard deviations. Uppercase letters indicate the interactive effect of stages × kapok that are significantly ($P < 0.05$) different from each other.

Stages	Kapok	N_o (mg kg^{-1})	k	R^2
Tillering	With	41.67	0.26	0.98
	Without	38.75	0.21	0.96
Booting	With	45.74	0.22	0.97
	Without	38.96	0.21	0.98
Heading	With	56.12	0.24	0.96
	Without	49.17	0.19	0.98
Maturity	With	63.42	0.29	0.98
	Without	52.65	0.26	0.99
Fallow	With	41.26	0.11	0.98
	Without	38.26	0.12	0.99

3.5 Soil microbial Diversity and Community Structure Composition under with kapok and without kapok systems

The study area in Changjiang County on Hainan Island harbors diverse soil microbial resources where *Bombax ceiba*–rice agroforestry is prevalent and well preserved. Bacterial communities were found dominated by phyla Pseudomonadota, Actinomycetota, Acidobacteriota, Myxococcota and Nitrospirota, together comprised roughly 75% of the communities across all rice growth stages (Fig. 3A), while accounted for > 1% in the relative abundance of bacterial groups in soil at different rice growth stages (Fig. 4A). Pseudomonadota were significantly more abundant at the maturity stage compared to other stages, making them dominant at phylum level, however, whereas Acidobacteriota exhibited non significantly lower abundance and were higher at the booting stage. In comparison to fallow stage, the relative abundance of Pseudomonadota and Actinobacteria showed an increasing trend at all other stages, however the difference was non-significant. The relative abundance of Myxococcota and

Verrucomicrobiota was relatively lower at all the stages in both the soils. For unclassified bacteria, the relative abundance showed minimal variation across all rice reproductive stages, suggesting the presence of numerous potential bacterial species in the kapok–rice agroforestry.

Fungal communities exhibited a more distinct classification compared to bacterial communities at the phylum level, with Bacillariophyta and Ascomycota particularly dominant at the heading, maturity and fallow stages, collectively accounted for more than 65% of the fungal communities across all rice growth stages (Fig. 3B). At phylum level among the fungal groups, Bacillariophyta, Ascomycota, Picozoa, Basidiomycota, Mucoromycota, Oomycota and Zoopagomycota were all exceeded 1% in their relative abundances in the different soil stages (Fig. 4B). Bacillariophyta showed an increasing trend whereas Ascomycota showed a relatively higher value at the maturity stage than other stages.

The α-diversity of fungal and bacterial communities across five rice growth stages was shown in Table 3. Bacterial Shannon and Simpson's diversity indices were generally similar to those of fungi. Bacterial Shannon in soils with kapok showed significant difference at maturity stage, compared with other stages. Simpson diversity index at the maturity (0.41 ± 0.01) was significantly ($P < 0.05$) higher than all the other rice growth stages. As for fungal community, fungal Shannon index at the maturity stage (1.51 ± 0.14) showed significant differences than other stages ($P < 0.05$), while non-significant differences were observed among other stages ($P > 0.05$), and Simpson index at maturity stage (0.49 ± 0.11) was also significantly higher than the other stages ($P < 0.05$), with a little or no significant differences among the other stages ($P > 0.05$). PCA analysis represented significant variations in the beta diversity patterns of both bacterial and fungal communities, different stages and soils with and without kapok. For bacteria and fungi, PCA plots revealed distinct bacterial and fungal communities in soils (Fig. 4B and 5B), explaining 60.48% & 20.78% and 45.85% & 14.70% of variations by PC1 and PC2, respectively.

Table 3
Values of Alpha diversity indices. Values are means ± standard deviations. Uppercase letters indicate the interactive effect of stages × kapok that are significantly ($P < 0.05$) different from each other.

Stages	Kapok	Shannon's Diversity Index		Simpson's Diversity index	
		Fungi	Bacteria	Fungi	Bacteria
Tillering	With	1.32 ± 0.09 ABC	1.31 ± 0.01 AB	0.42 ± 0.06 ABC	0.35 ± 0.02 ABC
	Without	1.09 ± 0.14 CD	1.30 ± 0.01 AB	0.22 ± 0.03 D	0.32 ± 0.03 C
Booting	With	1.46 ± 0.02 AB	1.38 ± 0.04 AB	0.41 ± 0.07 AB	0.39 ± 0.01 AB
	Without	1.14 ± 0.08 CD	1.32 ± 0.02 AB	0.22 ± 0.03 D	0.36 ± 0.01 ABC
Heading	With	1.46 ± 0.17 AB	1.46 ± 0.03 A	0.42 ± 0.09 AB	0.41 ± 0.01 A
	Without	1.22 ± 0.22 BCD	1.39 ± 0.04 AB	0.25 ± 0.02 D	0.36 ± 0.02 ABC
Maturity	With	1.51 ± 0.14 A	1.47 ± 0.09 A	0.49 ± 0.11 A	0.41 ± 0.01 A
	Without	1.25 ± 0.12 ABC	1.39 ± 0.05 AB	0.36 ± 0.09 ABC	0.39 ± 0.04 AB
Fallow	With	1.26 ± 0.19 ABC	1.31 ± 0.19 AB	0.36 ± 0.11 ABC	0.36 ± 0.02 ABC
	Without	1.01 ± 0.21 E	1.26 ± 0.13 B	0.20 ± 0.09 E	0.33 ± 0.01 BC

3.6 The relative contribution of soil properties, microbial biomass, microbial community and enzyme activities to net N mineralization variation in both the soils

Figure 6 shows the proportional contributions based on results from variation partitioning analysis (VPA), explaining the contribution of biotic factors (SMBC, SMBN, microbial communities, LAP and NAG), abiotic factors (SOM, total K, total P, total N and available N) and N content (NO_3^--N and NH_4^+-N) to the net N mineralization in both the soils with kapok and without kapok. The biotic factors and N contents explained most of the variations related to net N mineralization based on the variance portioning analysis. Overall, VPA explained 55.72% of the variations in soil with kapok, whereas it explained 51.29% of the total variation in soils without kapok.

4. Discussion

4.1 Effects of kapok trees on soil nutrient content

Soil properties exert a more substantial influence in elucidating nutrient cycling and community alterations within the soil. The results revealed that soils with kapok trees significantly affected soil nutrient properties, with nutrient concentrations being markedly higher compared with those of soils without kapok trees across all rice reproductive stages (Table 1). Agroforestry systems that combine trees with rice cultivation often outperform rice mono-cropping in improving soil nutrient status and

stimulating enzyme activities (Duanyuan et al. 2023; Xu et al. 2024; Al-Jawahry and Almoussaw 2025). These benefits are largely driven by greater plant diversity, which supports favorable soil physicochemical conditions and enhances microbial functioning (Nasar et al. 2024; You et al. 2024). A key pathway is the enrichment of soil organic matter and carbon pools. In particular, increased crop and vegetation diversity in agroforestry can promote the accumulation and sequestration of soil organic carbon (SOC) and nitrogen (N), with these effects typically becoming more pronounced as the system matures over time (Sun et al. 2024). These elevated soil nutrients at distance D0 may result from a collective effect from features, for example the leaf litter and flowers of mature *B. ceiba* tree/trees (Pardon et al. 2017; Wang et al. 2022). The blooming of *B. ceiba*, in conjunction with rice transplanting and complete shedding of flowers at the rice reproductive stage (Xiang and Ren 2019; Xiang et al. 2022), could promote the buildup of organic matter in soil and increase the availability of other nutrients (Pardon et al. 2017; Hassan 2018). Furthermore, the observed impact of soil organic matter and nitrogen may result from long term N fertilization throughout the history of cultivation (Zhang et al., 2020). All soil nutrient properties were strongly influenced by different rice growth stages, with nutrient cycling by leaf litter in the cocoa agro-forestry system exhibiting seasonal variation (Asigbaase et al. 2021). SOC also varies with the rice developmental stages in paddy and upland rotation system (Yang et al. 2019), indicating that temporal/seasonal dynamics strongly influence ecological processes in the *B. ceiba*–rice agroforestry system. We observed significantly higher SOM, as well as available and total nutrient contents at maturity stage of rice. These findings may be linked to the varying nutrient absorption patterns of rice at different growth stages.

4.2 Changes in soil extracellular enzymes and microbial biomass in response to kapok trees

In our study, SMBC and SMBN were observed to be at their peak during the crop maturity stage and in soils with kapok trees (Fig. 1). Agroforestry systems that combine tree plantations with rice cultivation substantially boost SMBC and SMBN compared to conventional monoculture rice farming (Sun et al. 2024; You et al. 2024). This improvement arises from several interconnected factors, including greater organic matter inputs from tree litter fall and root residues, enhanced plant species diversity, beneficial root exudates released into the soil, and overall more favorable soil microenvironments (Sun et al. 2024). These conditions collectively foster a more abundant, diverse, and active soil microbial community, which plays a vital role in maintaining long-term soil fertility, efficient nutrient cycling, and overall ecosystem health (Lu et al. 2023). In soils with kapok tress, the integration of trees with rice not only increases the quantity and variety of organic substrates available to microorganisms but also promotes better soil structure and resource use, leading to significantly higher levels of SMBC and SMBN (Nasar et al. 2024). This microbial enhancement serves as a key indicator of improved soil quality and more sustainable nutrient dynamics in diversified agroforestry systems.

Soil enzymes are widely used as sensitive indicators of soil health because they reflect the strength of biogeochemical reactions and nutrient cycling processes (Xu et al. 2024; Xiao et al. 2025). Agroforestry systems often stimulate enzyme activities by improving soil conditions and biological functioning. For

instance, rubber-based agroforestry has been reported to increase soil extracellular enzyme activities (EEAs), which can intensify nutrient transformations, although such stimulation may also increase microbial nutrient limitations (Xu et al. 2024). Likewise, integrating fruit trees into tea plantations has been associated with higher nutrient availability and improved soil aggregate stability, both of which are commonly linked to enhanced enzymatic activity (Duan et al. 2024). In addition, straw mulching in Camellia oleifera–cassia intercropping systems has been shown to raise soil carbon and nitrogen levels and promote key enzymes such as urease, sucrase, alkaline phosphatase, and catalase, particularly in surface layers (Duanyuan et al. 2023). These improvements are generally attributed to greater organic inputs from tree litter and crop residues, which enrich soil substrates and support a more diverse and active microbial community (Tang et al. 2024). Plant species also differ in the quantity and composition of root exudates, creating heterogeneous microhabitats and resource pools that further shape belowground biodiversity. Through decomposition and nutrient transformations mediated by microorganisms, extracellular enzymes act as catalysts that drive organic matter turnover and nutrient release (Li et al., 2024).

4.3 Gross N mineralization as affected by kapok trees

The incorporation of trees into rice fields, as commonly practiced in agroforestry systems, markedly alters nitrogen mineralization processes compared to traditional monoculture rice cultivation without trees (Fig. 2). These changes arise from intricate tree-soil interactions that modify the soil's physical structure, chemical composition, and biological activity, ultimately influencing the release and availability of nitrogen for rice plant uptake (Mahmud et al. 2024). Nitrogen mineralization refers to the microbial transformation of organic nitrogen compounds in the soil into inorganic, plant-accessible forms mainly ammonium (NH_4^+) and nitrate (NO_3^-) a key process that supports soil fertility and enhances crop growth and yield (Wang et al. 2024). The rate and efficiency of this mineralization are governed by several key factors, including the C:N ratio of organic residues, the level of soil microbial activity, as well as environmental conditions such as temperature and soil moisture. Trees in agroforestry systems enhance soil nitrogen mineralization through multiple synergistic mechanisms (Fig. 7). They supply substantial organic matter via litter fall, root exudates, and fine root decomposition, providing carbon and nitrogen substrates that fuel microbial activity, with lower C:N ratios favoring net nitrogen release (Han et al. 2025). This diverse organic input also stimulates a more active and diverse soil microbial community, particularly in the rhizosphere, accelerating decomposition and nitrogen transformation. Additionally, trees improve soil structure and porosity, promoting better aeration, water infiltration, and favorable microclimates for aerobic microbial processes (Rumeau et al. 2024). Their deep root systems act as a nutrient safety net by scavenging and redistributing mineral nitrogen from deeper layers back to the surface via litter, reducing leaching losses and enriching the overall nitrogen pool (Erktan et al. 2020). Finally, trees can influence the ammonium-to-nitrate balance in the soil, modulating nitrogen availability and potential losses (Guerrieri et al. 2024). These combined effects significantly boost nitrogen mineralization rates and availability in soils with kapok, compared to monoculture systems without kapok trees. In summary, integrating kapok trees into rice fields can significantly enhance nitrogen mineralization by increasing organic matter supply, stimulating soil microbial activity, and improving soil

physical properties. However, the extent and effectiveness of these benefits vary widely depending on tree species, tree age, local soil conditions, and prevailing environmental factors (Amare et al. 2024). Careful evaluation of these interacting variables is essential to maximize nutrient cycling efficiency and rice yield in agroforestry systems.

4.4 Influence of kapok trees on microbial community

Kapok trees significantly alter the abundance and composition of soil microbial communities in soils compared to the soils having no kapok tress (Fig. 4). This effect arises from multiple interacting factors, including organic matter addition, nutrient cycling, changes in soil physical structure, and direct plant-microbe interactions in the rhizosphere (Li et al., 2024; Wan et al., 2022). Trees supply substantial organic carbon through leaf litter, root residues, and exudates, which serve as key energy sources fueling microbial growth and activity (Zhou et al. 2022). Root exudates, in particular, act as a diverse mix of chemical signals and nutrients that selectively shape microbial populations in the rhizosphere, the nutrient-rich zone immediately surrounding roots leading to higher microbial density and activity (Zhou et al. 2022). Previous research indicates that microbial communities vary distinctly along the soil-root continuum, influenced by root anatomy, soil characteristics, and patterns of root exudation, resulting in clear shifts in community structure from bulk soil to the root interior (Li et al., 2024; Zhou et al., 2022).

In addition, tree species and overall vegetation type strongly shape distinct soil microbial communities. Greater tree diversity enhances linkages between microbial communities and ecosystem functions, boosting microbial biomass and diversity, as also observed in subtropical forests (Beugnon et al. 2021; Li et al. 2023). Additionally, trees improve soil physical properties; such as structure and moisture retention creating more favorable conditions for microbial growth (Erktan et al. 2020; Pathan et al. 2021). Stable soil aggregates physically shield soil organic matter and microbial biomass from oxidative stress, supporting long-term carbon storage and microbial persistence. These physical changes in soil structure both influence and result from biological interactions, offering a potential framework for understanding microbial trophic dynamics at small scales (Pathan et al. 2021). The presence of kapok trees in paddy soils further shape the functional traits of microbial communities through differences in litter quality, root exudates, and associations with mycorrhizal fungi (Prescott and Grayston 2013). Arbuscular mycorrhizal (AM) fungi, which form symbiotic partnerships with plant roots, extend nutrient and water uptake, particularly for soil P, and significantly boost microbial populations: studies show increases of 6.41% in total microbial biomass, 20.51% in bacterial biomass, and 45.14% in fungal biomass following AM inoculation (Gou et al. 2024). Overall, the greater microbial abundance and diversity in kapok-integrated paddy soils compared to soils without kapok, result from increased organic matter supply, better soil physical and chemical conditions, and the promotion of beneficial plant-microbe symbiotic relationships, creating a more robust and active soil microbial ecosystem.

5. Conclusions

This study highlights the transformative potential of the traditional kapok–rice agroforestry system with *B. ceiba* in western Hainan as a sustainable, nature-based solution for improving soil health and rice

productivity. Compared to rice-monoculture, the presence of *B. ceiba* significantly enhanced soil nutrients (SOM up to 216%, available N by 63%, available P by 133%, available K by 93% at maturity), increased SMBC (397.17 mg kg^{-1}) and SMBN (362.40 mg kg^{-1}), elevated N-acquiring enzyme activities (NAG up to 88.66 μmol h^{-1} g^{-1}, LAP up to 25.21 μmol h^{-1} g^{-1}), and boosted nitrogen mineralization potential (N_o up to 63.42 mg kg^{-1}) and cumulative mineralized N (67.46 mg kg^{-1} over 90 days). These results, driven by organic matter inputs, improved soil structure, and favorable shifts in microbial communities (dominated by Pseudomonadota, Actinomycetota, Ascomycota, and Bacillariophyta) due to *B. cieba*, create nutrient-rich hotspots that enhance N retention and availability across rice growth stages. Consequently, total mineralized N and mineralization potential (N_o) were highest at maturity and highest in soil with kapok trees, while the mineralization rate constant (k) increased across different rice reproductive stages. Future studies should focus on optimizing tree management, conducting long-term trials, and assessing economic viability to support wider adoption and maximize ecological and agronomic benefits.

Declarations

Conflict of interests

All the authors declared that there is no conflict of interest.

Author Contribution

S.A. : Conceptualization, Methodology, Investigation, Formal analysis, Writing – Original Draft. W.-Q.X. : Investigation, Formal analysis, Data curation, Visualization. M.-X.R. : Conceptualization, Resources, Supervision, Funding acquisition, Writing – Review & Editing. W.A. : Methodology (bioinformatics), Software, Formal analysis, Writing – Review & Editing.

Acknowledgements

This research work was supported by the National Natural Science Foundation of China (42371054 and 32401285) and the Provincial Postdoctoral Science Fund (Science and Technology) (RZ2500004811).

References

1. Abid AA, Yu S, Zou X, et al (2024) Unraveling nitrogen loss in paddy soils: A study of anaerobic nitrogen transformation in response to various irrigation practice. Environ Res. https://doi.org/10.1016/j.envres.2024.118693

2. Al-Jawahry HM, Almoussaw ZA (2025) Exploring the Potential of Agroforestry to Improve Soil Fertility and Crop Yields. SHS Web Conf. https://doi.org/10.1051/shsconf/202521601050
3. Amare S, Haile M, Birhane E (2024) Changes in Ammonium-to-Nitrate Ratio along Faidherbia albida Tree Age Gradients in Arenosols. Nitrogen (Switzerland). https://doi.org/10.3390/nitrogen5030035
4. Asigbaase M, Dawoe E, Lomax BH, Sjogersten S (2021) Temporal changes in litterfall and potential nutrient return in cocoa agroforestry systems under organic and conventional management, Ghana. Heliyon. https://doi.org/10.1016/j.heliyon.2021.e08051
5. Beugnon R, Du J, Cesarz S, et al (2021) Tree diversity and soil chemical properties drive the linkages between soil microbial community and ecosystem functioning. ISME Commun. https://doi.org/10.1038/s43705-021-00040-0
6. Beule L, Corre MD, Schmidt M, et al (2019) Conversion of monoculture cropland and open grassland to agroforestry alters the abundance of soil bacteria, fungi and soil-N-cycling genes. PLoS One. https://doi.org/10.1371/journal.pone.0218779
7. Black CA, Evans DD, White JL, et al (2016) Methods of soil analysis: Part 2. Methods Soil Anal Part 2 Chem Microbiol Prop 1–1572. https://doi.org/10.2134/agronmonogr9.2.2ed
8. Duan Y, Wang G, Liang L, et al (2024) Intercropping fruit trees in tea plantation improves soil properties and the formation of tea quality components. Plant Physiol Biochem. https://doi.org/10.1016/j.plaphy.2024.108574
9. Duanyuan H, Zhou T, He Z, et al (2023) Effects of Straw Mulching on Soil Properties and Enzyme Activities of Camellia oleifera–Cassia Intercropping Agroforestry Systems. Plants. https://doi.org/10.3390/plants12173046
10. Elrys AS, Uwiragiye Y, Zhang Y, et al (2023) Expanding agroforestry can increase nitrate retention and mitigate the global impact of a leaky nitrogen cycle in croplands. Nat Food. https://doi.org/10.1038/s43016-022-00657-x
11. Erktan A, Or D, Scheu S (2020) The physical structure of soil: Determinant and consequence of trophic interactions. Soil Biol. Biochem.
12. Gou X, Kong W, Sadowsky MJ, et al (2024) Global responses of soil bacteria and fungi to inoculation with arbuscular mycorrhizal fungi. Catena. https://doi.org/10.1016/j.catena.2024.107817
13. Guerrieri R, Cáliz J, Mattana S, et al (2024) Substantial contribution of tree canopy nitrifiers to nitrogen fluxes in European forests. Nat Geosci. https://doi.org/10.1038/s41561-023-01364-3
14. Guo JH, Liu XJ, Zhang Y, et al (2010) Significant acidification in major chinese croplands. Science (80-) 327:1008–1010. https://doi.org/10.1126/science.1182570
15. Han S, Miao X, Zhang Y, Sun H (2025) Effects of Mixed Addition of Fraxinus mandshurica Rupr. and Larix gmelinii (Rupr.) Kuzen. Litter on Nitrogen Mineralization in Dark Brown Soil of Northeast China. Forests. https://doi.org/10.3390/f16050842
16. Hassan MO (2018) Leaf litter of Bombax ceiba L. threatens plant cover and floristic diversity in a new urban ecosystem. Flora Morphol Distrib Funct Ecol Plants. https://doi.org/10.1016/j.flora.2018.03.004

17. Hoque MB, Oyshi TH, Hannan MA, et al (2025) Plant science: Pioneering sustainable solutions for global challenges. Probe - Plant Anim Sci. https://doi.org/10.18686/ppas2266
18. Huang C, Butterly CR, Moody D, Pourkheirandish M (2022) Mini review: Targeting below-ground plant performance to improve nitrogen use efficiency (NUE) in barley. Front. Genet.
19. Islam KK, Saifullah M, Mahboob MG, et al (2024) Restoring Soil Fertility, Productivity and Biodiversity through Participatory Agroforestry: Evidence from Madhupur Sal Forest, Bangladesh. Land. https://doi.org/10.3390/land13030326
20. Jose S (2009) Agroforestry for ecosystem services and environmental benefits: An overview. Agrofor. Syst.
21. Khan MN, Huang J, shah A, et al (2022) Mitigation of greenhouse gas emissions from a red acidic soil by using magnesium-modified wheat straw biochar. Environ Res. https://doi.org/10.1016/j.envres.2021.111879
22. Kim DG, Isaac ME (2022) Nitrogen dynamics in agroforestry systems. A review. Agron. Sustain. Dev.
23. Li J, Wu J, Yu J, et al (2024a) Soil enzyme activity and stoichiometry in response to precipitation changes in terrestrial ecosystems. Soil Biol Biochem. https://doi.org/10.1016/j.soilbio.2024.109321
24. Li T, Hong X, Liu S, et al (2022) Cropland degradation and nutrient overload on Hainan Island: A review and synthesis. Environ. Pollut.
25. Li Y, Qian Z, Li D (2023) Effects of tree diversity on soil microbial community in a subtropical forest in Southwest China. Eur J Soil Biol. https://doi.org/10.1016/j.ejsobi.2023.103490
26. Li YZ, Bao XL, Tang SX, et al (2024b) Toward soil carbon storage: The influence of parent material and vegetation on profile-scale microbial community structure and necromass accumulation. Soil Biol Biochem. https://doi.org/10.1016/j.soilbio.2024.109399
27. Lu P, Zhao C, Yin W, et al (2023) Microbial Community Shifts with Soil Properties and Enzyme Activities in Inter-/Mono-Cropping Systems in Response to Tillage. Agronomy. https://doi.org/10.3390/agronomy13112707
28. Mahmud M, Bertrand I, Bazot S, et al (2024) Assessing Nutrient Safety Net and Crop Yield in a Mediterranean Agroforestry Using 15N Labelling Experiment. J Soil Sci Plant Nutr. https://doi.org/10.1007/s42729-024-01845-3
29. Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta 27:31–36. https://doi.org/10.1016/S0003-2670(00)88444-5
30. Nair PKR (2012) Carbon sequestration studies in agroforestry systems: A reality-check. Agrofor Syst. https://doi.org/10.1007/s10457-011-9434-z
31. Nasar J, Ahmad M, Gitari H, et al (2024) Maize/soybean intercropping increases nutrient uptake, crop yield and modifies soil physio-chemical characteristics and enzymatic activities in the subtropical humid region based in Southwest China. BMC Plant Biol. https://doi.org/10.1186/s12870-024-05061-0

32. Niguse G, Iticha B, Kebede G, Chimdi A (2022) Contribution of coffee plants to carbon sequestration in agroforestry systems of Southwestern Ethiopia. J Agric Sci. https://doi.org/10.1017/S0021859622000624
33. Olsen, S.R., Sommers LE (1982) Methods of Soil Analysis, Part 2, Chemical and Microbial Properties Agronomy Society of America, Agronomy Monograph 9, Madison, Wisconsin. Soil Sci Soc Am Inc Madison, WI, USA 403–430.
34. Pardon P, Reubens B, Reheul D, et al (2017) Trees increase soil organic carbon and nutrient availability in temperate agroforestry systems. Agric Ecosyst Environ. https://doi.org/10.1016/j.agee.2017.06.018
35. Parkinson JA, Allen SE (1975) A wet oxidation procedure suitable for the determination of nitrogen and mineral nutrients in biological material. Commun Soil Sci Plant Anal. https://doi.org/10.1080/00103627509366539
36. Pathan SI, Arfaioli P, Ceccherini MT, et al (2021) Physical protection of extracellular and intracellular DNA in soil aggregates against simulated natural oxidative processes. Appl Soil Ecol. https://doi.org/10.1016/j.apsoil.2021.104002
37. Prescott CE, Grayston SJ (2013) Tree species influence on microbial communities in litter and soil: Current knowledge and research needs. For Ecol Manage. https://doi.org/10.1016/j.foreco.2013.02.034
38. Qi D, Wu Z, Yang C, et al (2023) Exploration and Practice of Rubber Based Agroforestry Complex Systems in China. Adv Biosci Biotechnol. https://doi.org/10.4236/abb.2023.1412033
39. Reynolds M, Tuberosa R, Mehta KM, et al (2015) Nitrogen use efficiency inwheat in bread wheat (T. NITROGEN USE EFFICIENCY IN BREAD BREEDING & GENE DISCOVERY. J Integr Agric
40. Rukun L (1999) Methods in soil agricultural chemical analysis: I. Analysis of chemical properties of soil and water. China Agric Sci Technol Press Beijing 7:139
41. Rumeau M, Sgouridis F, MacKenzie R, et al (2024) The role of rhizosphere in enhancing N availability in a mature temperate forest under elevated CO2. Soil Biol Biochem. https://doi.org/10.1016/j.soilbio.2024.109537
42. Stanford G, Smith SJ (1972) Nitrogen Mineralization Potentials of Soils. Soil Sci Soc Am J 36:NP-NP. https://doi.org/10.2136/sssaj1972.03615995003600030049x
43. Sun T, Zhou J, Fu Y, et al (2024) Soil nitrogen availability mediates the positive effects of intercropping on soil organic carbon at global scales. Soil Tillage Res. https://doi.org/10.1016/j.still.2024.106063
44. Tang Z, He N, Zhang L, et al (2024) Straw and Biochar Application Alters the Structure of Rhizosphere Microbial Communities in Direct-Seeded Rice (Oryza sativa L.) Paddies. Agronomy. https://doi.org/10.3390/agronomy14020316
45. Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:703–707. https://doi.org/10.1016/0038-0717(87)90052-6

46. Voroney P, Brookes PC, Beyaert RP (2008) Soil Microbial Biomass C, N, P and S. | Directory of scientists and professionals. Soil sampling and methods of analysis
47. Wan X, Yu Z, Wang M, et al (2022) Functional trait variation and community-weighted means of tree traits can alter soil microbial biomass and community composition. Soil Biol Biochem. https://doi.org/10.1016/j.soilbio.2022.108715
48. Wang WJ, Wen J, Xiang WQ, et al (2022) Soil bacterial and fungal communities respond differently to Bombax ceiba (Malvaceae) during reproductive stages of rice in a traditional agroforestry system. Plant Soil. https://doi.org/10.1007/s11104-022-05542-x
49. Wang Y, Tang G, Fu W, et al (2024) Rice Yield and Nitrogen Use Efficiency: Different Responses to Soil Organic Matter between Early and Late Rice. J Soil Sci Plant Nutr. https://doi.org/10.1007/s42729-024-01896-6
50. Wei JZ, Wang DF, Wei ZY, Qi ZP (2013) Temporal and spatial distribution of soil pH in arable land of Changjiang, Hainan. Chin J Trop Crop 34:413–417
51. Wen S, Yao W, Tian B, et al (2024) Spatiotemporal dynamic of rice production and its carbon footprint in Hainan, China: Implications for food security and environmental sustainability. J Environ Qual. https://doi.org/10.1002/jeq2.20590
52. Xiang W, Ren M (2019) Adaptive significance of yellow flowered Bombax ceiba (Malvaceae). Biodivers Sci. https://doi.org/10.17520/biods.2019003
53. Xiang W, Wang W, Ren M (2023) Traditional biodiversity knowledge in Bombax ceiba cultures: Inheritance and utilization. Biodivers. Sci.
54. Xiang WQ, Zhang JR, Tang L, Ren MX (2022) Filament union provides landing site for birds and increase pollen removal and deposition in an ornithophilous species. Flora Morphol Distrib Funct Ecol Plants. https://doi.org/10.1016/j.flora.2022.152025
55. Xiao L, Min X, Li Z, Li P (2025) Kinetic and Thermodynamic Characteristics of Soil Enzymes in Pure and Mixed Forest Samples on the Loess Plateau of China. J Plant Nutr Soil Sci. https://doi.org/10.1002/jpln.202400242
56. Xu W, Zhang Y, Tahir A, et al (2024) Rubber-Based Agroforestry Ecosystems Enhance Soil Enzyme Activity but Exacerbate Microbial Nutrient Limitations. Forests. https://doi.org/10.3390/f15101827
57. Yang BJ, Sun DP, Zhang YR, et al (2019) Effects of different paddy-upland multiple cropping rotation systems on soil organic carbon and its fractions in paddy field. Chinese J Appl Ecol. https://doi.org/10.13287/j.1001-9332.201902.021
58. You Y, Wang L, Liu X, et al (2024) Interspecific plant interaction structures the microbiomes of poplar-soil interface to alter nutrient cycling and utilization. Microbiol Spectr. https://doi.org/10.1128/spectrum.03368-23
59. Young (2012) Pingali, P. (2012). Green Revolution: Impacts, limits, and the path ahead. Proceedings of the National Academy of Sciences, 109(31), 12302–12308. https://doi.org/10.1073/pnas.09129531. Экономика Региона

60. Yule CM (2010) Loss of biodiversity and ecosystem functioning in Indo-Malayan peat swamp forests. Biodivers Conserv. https://doi.org/10.1007/s10531-008-9510-5
61. Zhang Q, Miao F, Wang Z, et al (2017) Effects of long-term fertilization management practices on soil microbial biomass in China's cropland: A meta-analysis. Agron J 109:1183–1195. https://doi.org/10.2134/agronj2016.09.0553
62. Zhang R, Mu Y, Li X, et al (2020) Response of the arbuscular mycorrhizal fungi diversity and community in maize and soybean rhizosphere soil and roots to intercropping systems with different nitrogen application rates. Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2020.139810
63. Zhou Y, Wei Y, Zhao Z, et al (2022) Microbial communities along the soil-root continuum are determined by root anatomical boundaries, soil properties, and root exudation. Soil Biol Biochem. https://doi.org/10.1016/j.soilbio.2022.108721

Figures

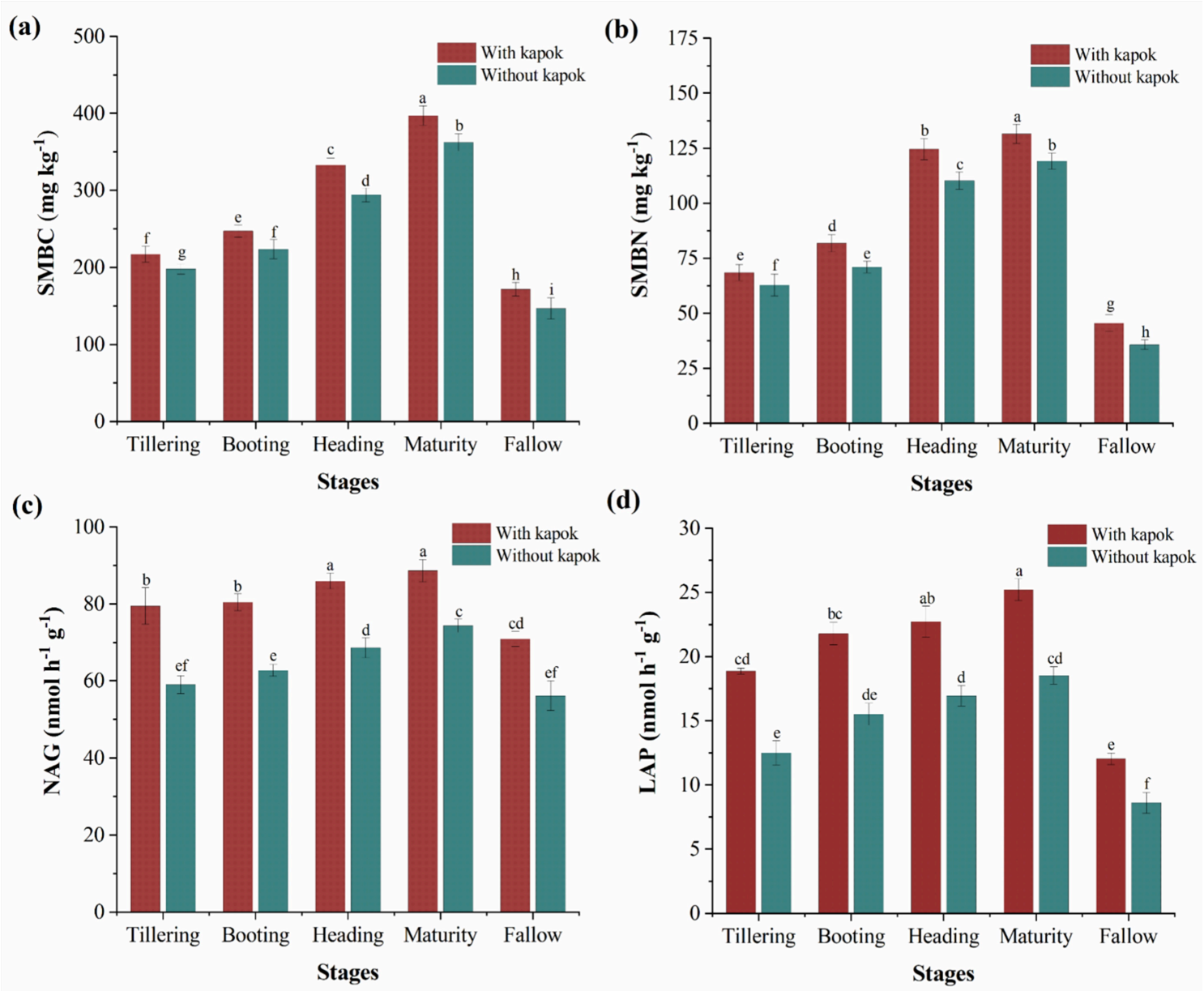


Figure 1

(a) Soil microbial biomass C (SMBC) and (b) Soil microbial biomass N (SMBN), extracellular enzymes assays, (c) N-Acetyl-β-D-glucosaminidase (NAG) and (d) leucine-aminopeptidase (LAP) in kapok and without kapok soils during rice reproductive stages.

Bars indicate standard error, n = 3. Lowercase letters indicate the interactive effect of stages × kapok that are significantly ($P < 0.05$) different from each other.

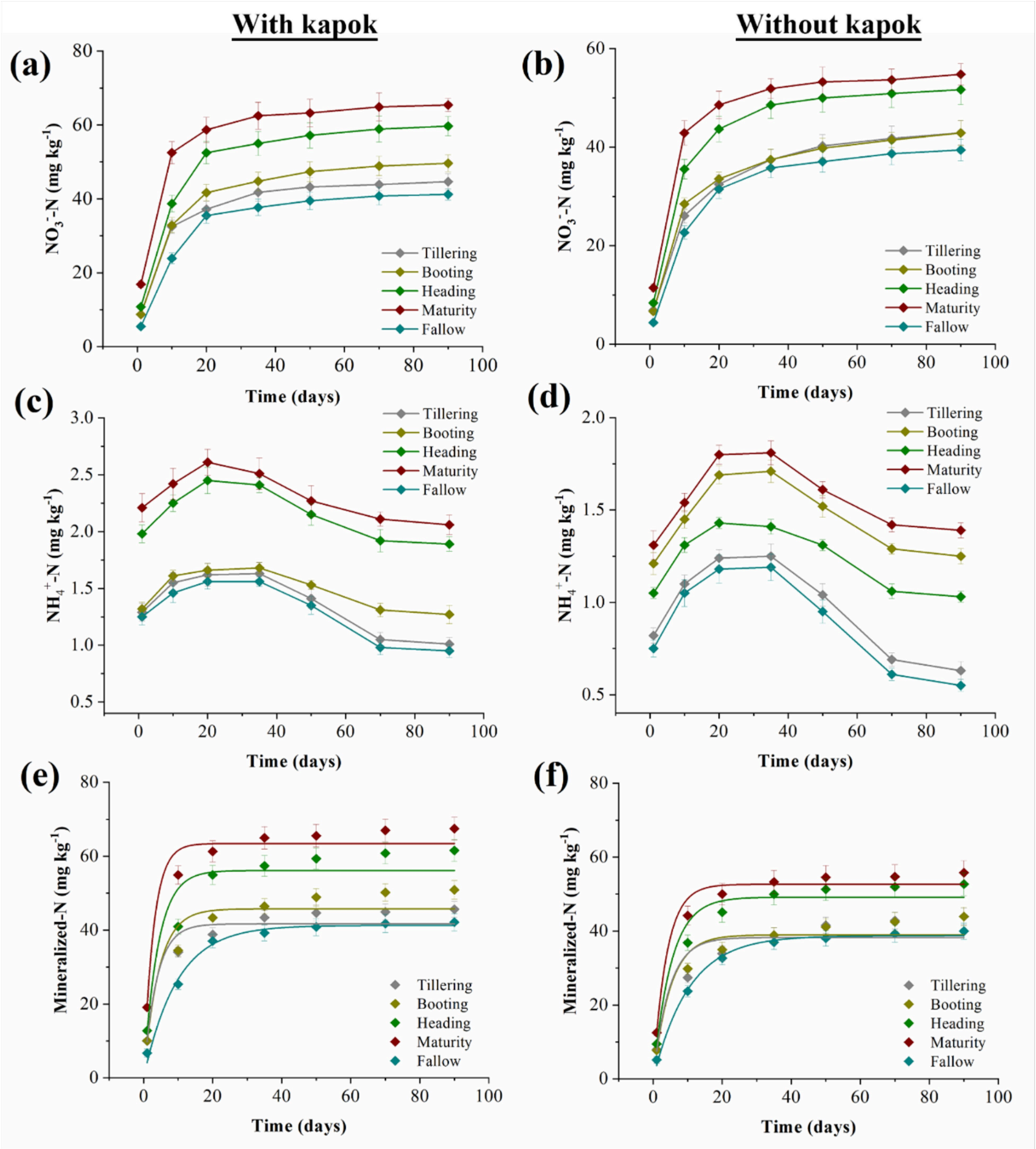


Figure 2

The concentrations of (a, b) NO_3^--N, (c, d) NH_4^+-N and (e, f) net N mineralization in soil during rice reproductive stages. The error bars denote the least significant difference values ($P < 0.05$) using the LSD comparison test.

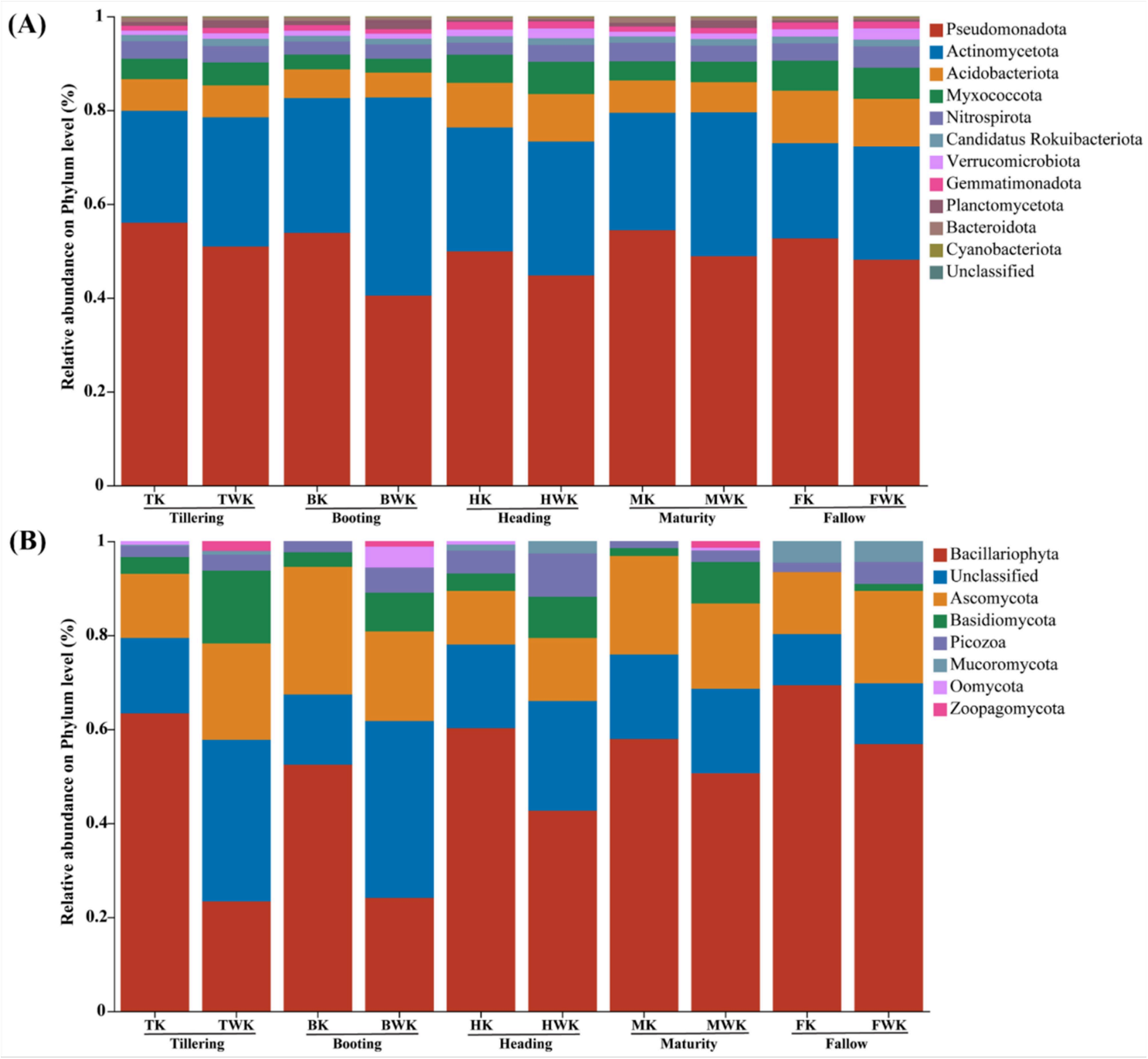


Figure 3

The relative abundance of dominant bacterial phyla (A) and fungal phyla (B) in soil during rice reproductive stages.

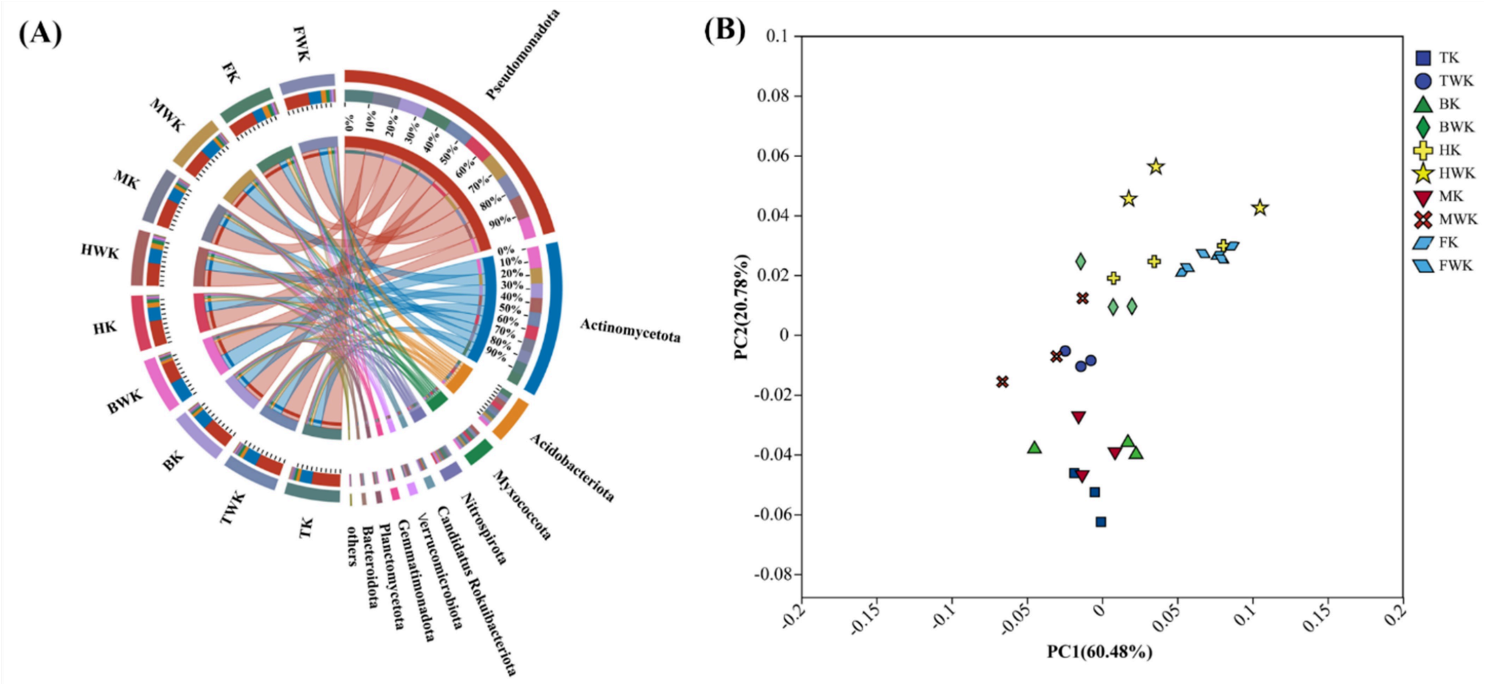


Figure 4

Circos of bacterial distribution with the top phylum abundance (A), and principal component analysis of dominant bacterial phyla (B) in soil during rice reproductive stages.

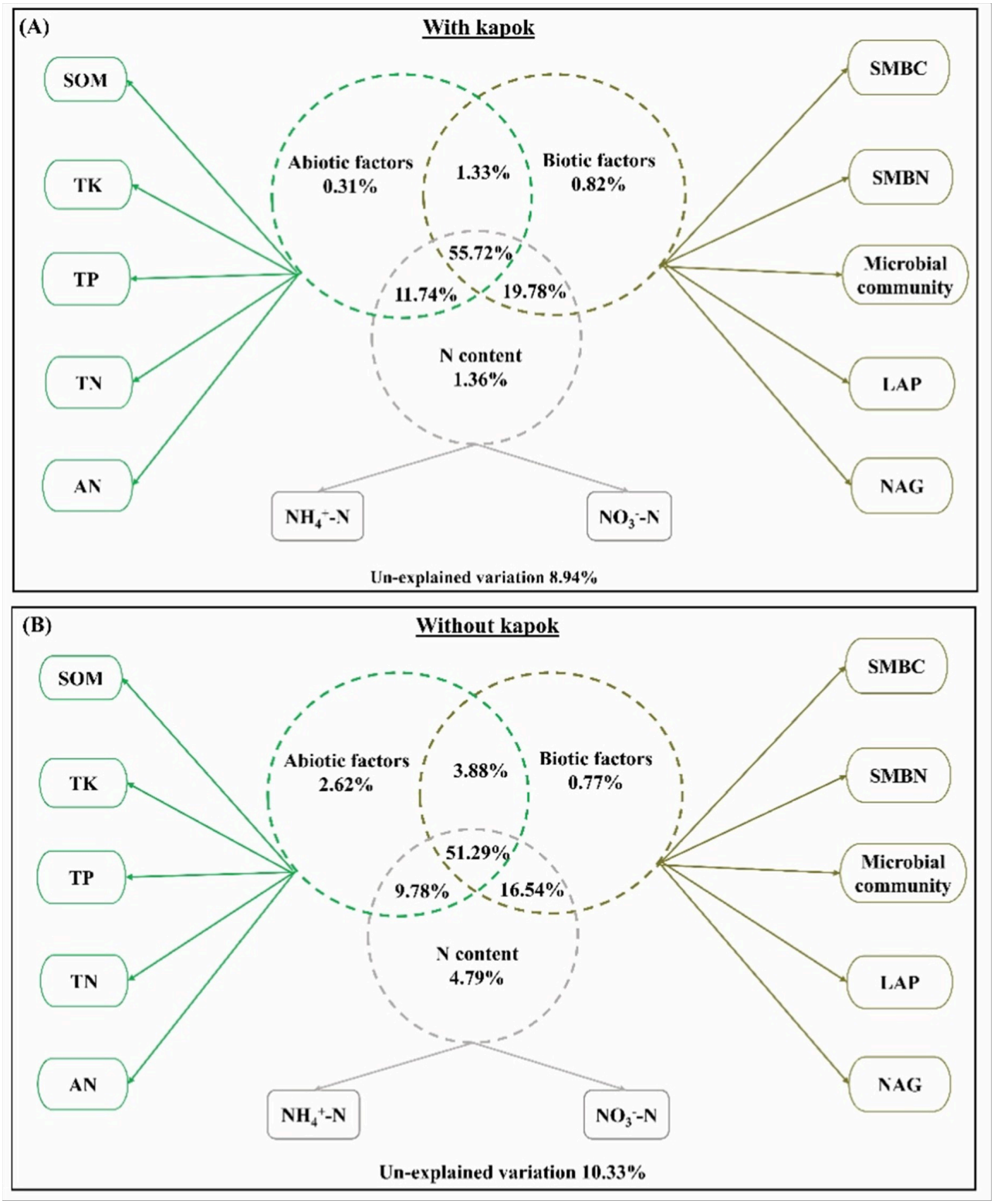


Figure 6

Venn diagrams showing the proportional contributions based on results from variation partitioning analysis (VPA), explaining the contribution of biotic factors, abiotic factors and N content to the net N mineralization in kapok (A) and without kapok soils (B). The effect of biotic factors (brown circle); the effect of abiotic factors (green circle); the effect of soil N content (gray circle); and their combined effects and respective variations explained are in areas overlapped in the circles.

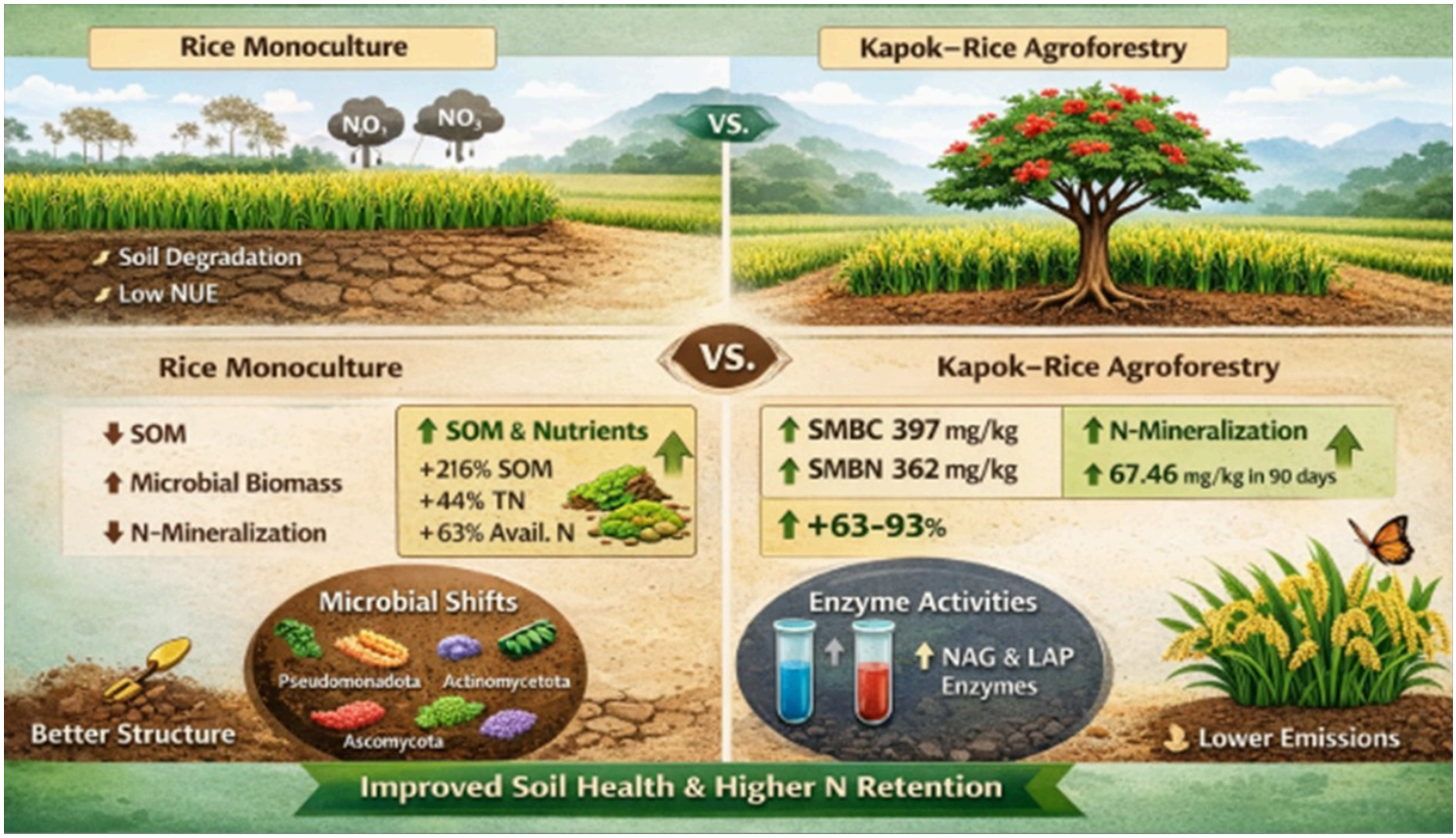


Figure 7

A conceptual diagram illustration of N transformation mechanisms in soil that how SOM, microbial biomass, extracellular enzymes and microbial community influence N transformation processes under traditional agroforestry systems.