



Rhizosphere abundant bacteria enhance buckwheat yield, while rare taxa regulate soil chemistry under diversified crop rotations

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ABSTRACT

Buckwheat is a fast-growing crop valued for its gluten-free grain, high rutin content, adaptability to suboptimal conditions, and minimal nutrient requirements, making it an ideal candidate for sustainable crop rotation systems. Crop rotations are known to profoundly shape the diversity, composition, and complexity of soil microbial communities, ultimately impacting the functioning and productivity of agroecosystems. While soil abundant and rare microbial communities serve distinct ecological roles, the specific effect of different rotation patterns and buckwheat species on these communities within the rhizosphere and their subsequent roles in agriculture functioning remain largely unknown. To address this gap, we conducted a three-year field trial to assess the relationships among soil properties, rhizosphere bacterial communities, and buckwheat yields under three rotation systems: wheat-buckwheat rotation (R1), continuous mono-buckwheat cropping (R2), and soybean-buckwheat rotation (R3), using both common and tartary buckwheat species. Compared to differences between buckwheat species, rotation systems more significantly influenced the attributes of both abundant and rare communities, soil chemical properties, and soil enzyme activities. Notably, R3 had a higher diversity of abundant taxa, enhanced complexity, cohesion, and robustness of abundant bacterial interactions, and a greater number of abundant biomarkers, relative to the monoculture practice in R2. This likely increased the resilience of abundant taxa in the diversified rotation system to stresses and facilitated belowground ecosystem functions, significantly contributing to higher buckwheat yield. In contrast, while R3 also increased diversity of rare taxa and altered their communities, these changes primarily affected soil chemical properties through modulating soil enzyme activities. These findings suggest that the attributes of abundant and rare taxa in the buckwheat rhizosphere, when intensified by the diversified rotation system, played distinct roles in different facets of agroecosystem functioning. Overall, our study highlights the importance of diversifying rotational diversity to bolster agricultural sustainability.

1. Introduction

The global demand for food is projected to increase significantly in the 21st century, due to population growth, changing dietary preferences, and economic development (Pinstripe-Andersen et al., 1999; Fróna et al., 2019). This increase is driving the expansion and intensification of agricultural systems worldwide (Pretty and Bharucha, 2014; Struik and Kuyper, 2017). In turn, these changes contribute to soil

degradation, increase the incidence of plant diseases, reduce crop yields and quality, ultimately resulting in substantial economic losses (Wang et al., 2024b). In response, sustainable farming practices are being developed to protect soil health while maintaining crop productivity and profitability. One such strategy is crop rotation, the sequential planting of different crops in the same field, which is increasingly recognized for its benefits to soil health and agroecosystem multifunctionality (Jiang et al., 2022b; Zhang et al., 2022b). Particularly, incorporating crops

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with distinct functional traits (e.g., perennial grasses, brassicas, cover crops, legumes, and grains) into crop sequences has been shown to significantly improve soil health compared to simplified crop rotations (Wick et al., 2017; Zhang et al., 2021). For example, Yang et al. (2024) presented that diversifying wheat–maize system with sweet potato, peanut, and soybean can stimulate soil microbial activities, enhance soil organic carbon (C) stocks, and increase crop yields by up to 38 %.

Soil microbial communities are pivotal in maintaining soil health by facilitating nutrient cycling, enhancing soil structure, and providing protection against pathogens and pests (Gao et al., 2024). Previous studies have investigated the effects of crop rotation systems on soil microbial abundance, diversity, composition, and community stability (Tripathi et al., 2017; Zhang et al., 2020, 2022a, 2022b; Liu et al., 2024). For example, Zhang et al. (2022b) reported that increased rotational diversity promoted the richness and abundance of keystone taxa within microbial networks, potentially strengthening ecological interconnections among soil, microorganisms, and crops, thereby enhancing C and nutrient cycling. However, there is still a significant knowledge gap concerning the relative contributions of different functionally partitioned groups of the microbial community, particularly the abundant and rare communities, to agroecosystem functions. Microbial communities exhibit uneven growth, activity, diversity, and composition, leading to an inequitable distribution of species abundance (Hanson et al., 2012). This distribution results in low diversity among abundant species and high diversity among rare species (Pedrós-Alió, 2012), suggesting their divergent roles in ecosystem functioning. For example, abundant communities, which constitute the majority of microbial biomass, predominantly drive biogeochemical cycling in agro-ecosystems (Jiao et al., 2022). In contrast, rare species, though low in abundance, are crucial for maintaining ecosystem function and stability due to their high diversity (Lynch and Neufeld, 2015). Interestingly, both groups respond differently to various agricultural management practices, such as the application of chemical fertilizers (Zhao et al., 2015) or manure (Liu et al., 2022b), and the restitution of crop residues (Liu et al., 2022a), highlighting the complexity of soil microbial community dynamics in response to different management practices. However, their responses and ecological roles under diversified crop rotations remain poorly understood, especially in the rhizosphere - a dynamic and biologically active interface where plant-microbe interactions are most intense (Kuzyakov and Blagodatskaya, 2015). Understanding how rhizosphere abundant and rare microbial communities contribute to soil function and crop productivity under different rotation systems is therefore critical for optimizing microbial management in sustainable agriculture.

Buckwheat (*Fagopyrum* spp.), an underutilized crop in the Polygonaceae family (Wijngaard and Arendt, 2006), is known for its remarkable adaptability to suboptimal soils and extreme environmental conditions. Buckwheat's rapid growth cycle, completing grain production within 60–70 days (Wijngaard and Arendt, 2006), makes it an ideal forecrop for enhancing the sustainability of agricultural systems and improving soil physical and biological properties through short-term crop rotations (Steinberga et al., 2012). Several studies have shown that using buckwheat as a forecrop can significantly enhance the production of subsequent crops such as potatoes (Han et al., 2021; Jiang et al., 2022a). Additionally, its botanical and physiological similarities to weeds, have made buckwheat effective in intercropping systems, where serves as a natural alternative to artificial fertilizers or pesticides (Lejins and Lejina, 2009). Recently, buckwheat has garnered attention for its essential nutrients and therapeutic compounds, such as dietary fiber, resistant starch, rutin, D-chiro-inositol, vitamins, and fagopyritols, which align with modern dietary preferences (Zhu, 2021; Jin et al., 2022; Zou et al., 2023). This renewed interest has sparked further research into its use for food production and nutritional applications (Jin et al., 2022; Zou et al., 2023). However, most studies have primarily focused on yield improvement through genomics, transcriptomics, and metabolomics approaches (Zargar et al., 2024). There remains a

compelling need to integrate breeding methodologies with sustainable agricultural practices to deepen our understanding of buckwheat biology and promote the development of commercially viable varieties. Of the primary species cultivated, common buckwheat (*F. esculentum*) and tartary buckwheat (*F. tatarium*) exhibit significant differences in key traits such as total flavonoids contents, with tartary buckwheat showing higher contents of rutin compared to common buckwheat (Lee et al., 2016; Zhou et al., 2018). Consequently, more research is urgently needed to explore how rotating different buckwheat species with crops possessing distinct functional traits can affect yield and agricultural sustainability.

This study aims to conduct a comprehensive exploration of the interplay among cropping systems, soil properties, and various soil microbial communities under different crop rotation systems and buckwheat species. Our primary objectives were to (1) assess the effects of various cropping systems on buckwheat yields; (2) investigate the distribution and assembly of both abundant and rare bacterial communities, along with their ecological roles under diverse crop rotation regimes; and (3) compare the contribution of abundant and rare communities to soil properties and agricultural productivity within different management systems. We hypothesized that while both crop rotation systems and buckwheat species would affect buckwheat yields, rotational patterns have a stronger effect on soil properties and microbial communities, with abundant bacterial communities playing a more pivotal role in increasing buckwheat yield and altering soil properties compared to rare communities.

2. Materials and methods

2.1. Experimental site and design

A field experiment was conducted from 2018 to 2020 at the research farm of Southwest University, Chongqing, China (29°76'N, 106°39'E; 350 m above sea level), featuring a subtropical monsoon climate. The site experienced annual mean temperatures of 20.9 °C in 2018, 20.1 °C in 2019, and 19.3 °C in 2020. Average annual rainfall, predominantly between June and September, was 92.2 mm in 2018, 85.8 mm in 2019, and 81.8 mm in 2020 (Fig. S1).

In mountainous regions studied, wheat and soybean represent the primary cash crops due to their economic value and local agronomic suitability. However, after harvesting wheat and soybean, there is typically a short fallow period before planting subsequent crops. To enhance regional agronomic practices and improve land-use efficiency during this interval, buckwheat, characterized by its short growth cycle and adaptability to marginal conditions, was introduced as a gap crop following wheat and soybean. Thus, three cropping systems were evaluated: a wheat-buckwheat rotation (R1), continuous buckwheat monocropping (R2), and a soybean-buckwheat rotation (R3). To assess varietal influences, the region's two primary cultivated buckwheat species, including common buckwheat and Tartary buckwheat, were introduced as the second factor. Consequently, this resulted in six treatments: wheat-common buckwheat (WC), wheat-tartary buckwheat (WT), common buckwheat-common buckwheat (BC), tartary buckwheat-tartary buckwheat (BT), soybean-common buckwheat (SC), and soybean-tartary buckwheat (ST), as shown in Fig. 1 A. This field trial was established using a randomized complete block design with three replications. Each treatment was randomly assigned to an individual experimental plot measuring 12 m² (3 m × 4 m) within each block, with 0.5 m alleys between adjacent plots. A buffer of 1.5 m was maintained between blocks to prevent cross-contamination. Seeds for wheat (Chuanmai 42), soybean (Xidou 8), and buckwheat (common buckwheat: Youqiao 2 and Ukrainian daliqiao; tartary buckwheat: Youqiao 1 and Juijiang) were sourced from the Institute of Characteristic Crops, College of Agronomy and Biotechnology, Southwest University. Autumn buckwheat was sown at the end of August and harvested at the beginning of December each year, and spring buckwheat in BC and BT was

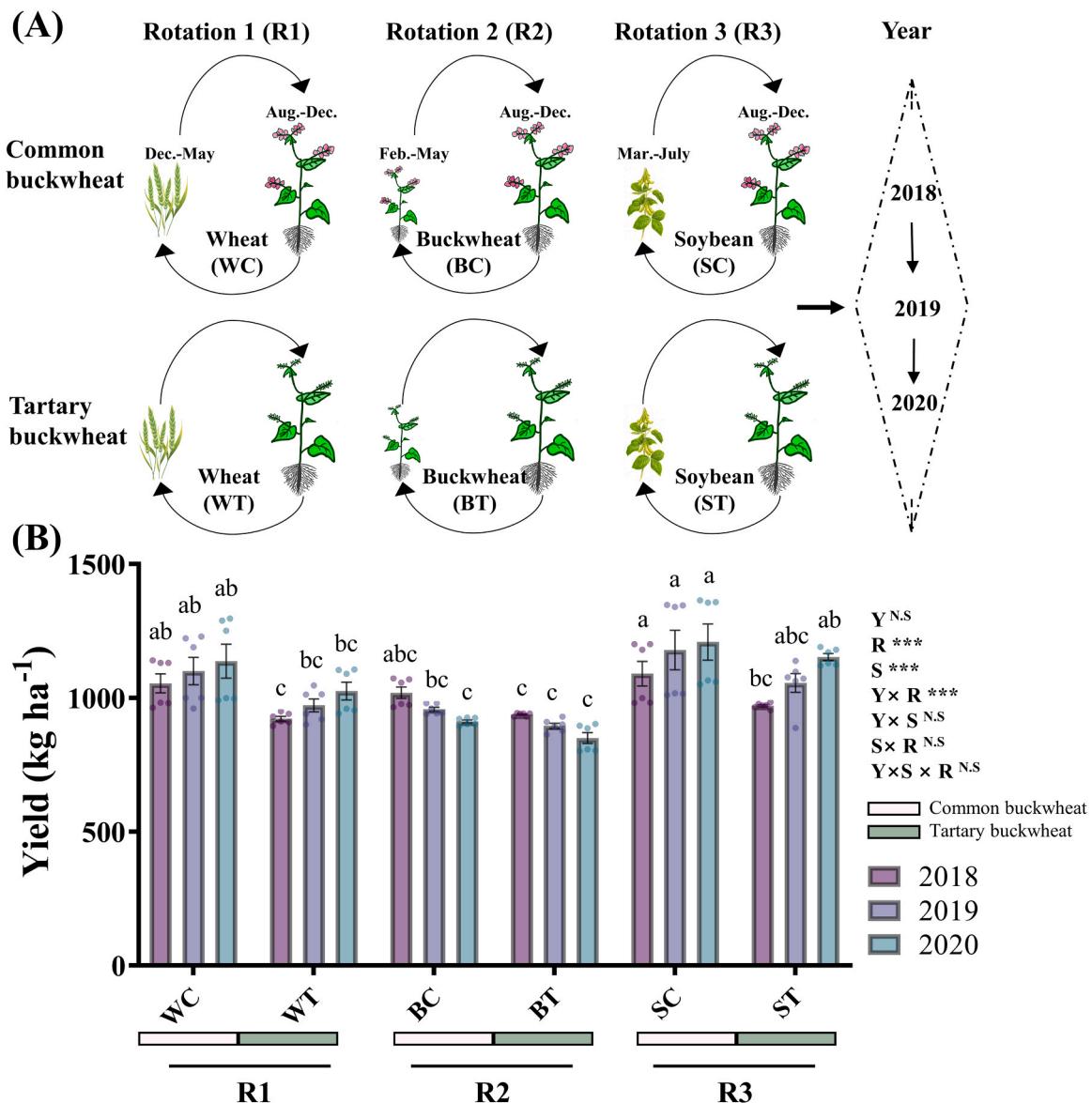


Fig. 1. Buckwheat yield under different rotation systems and species. (A) Schematic diagram of the experimental design. (B) Yield of buckwheat across different cropping management practices. The effects of buckwheat species, rotation systems, and sampling years on buckwheat yield were analyzed using a three-way ANOVA. Values represent mean of six replicates, with error bars indicating standard error. ***denotes significance at $P < 0.001$. N.S. indicates no significant effects. Lowercase letters above bars represent significant differences among treatments, as determined by Tukey's HSD test. Y: year; R: rotation; S: species.

sown in late February and harvested in late May. Soybeans in SC and ST were sown in late March and harvested in early July each year, and wheat in WC and WT was planted in mid-December and harvested in mid-May the following year (Fig. 1 A). The soil at the research site was classified as sandy loam with an organic matter (OM) content of 18.54 g kg^{-1} , a pH of 5.0, available potassium (AK) content of 69.9 mg kg^{-1} , total phosphate (TP) of 7.31 %, total potassium (TK) contents of 9.64 %, total nitrogen (TN) content of 11.59 %, and alkali nitrogen (Alkali N) of 18.9 mg kg^{-1} . Following local farming practices, an integrated fertilizer (15 % N, 15 % P_2O_5 , 15 % K_2O) at a rate of 450 kg ha^{-1} was applied to all crops before sowing in each experimental year (2018–2020).

2.2. Sample collection and analyses

2.2.1. Yield analysis

At the maturity stage from 2018 to 2020, defined as when 70–80 % of buckwheat grains in the field have matured each year, crops from all

blocks were harvested. After the crops were air dried, the yield for each plot was measured by threshing and converted into yield per hectare. Simultaneously, grain number, grain weight, and 1000-grain weight were determined from 5 representative buckwheat plants from each plot across all treatments to ensure consistent and comparable data collection.

2.2.2. Soil sampling and chemical properties analyses

In October 2019, buckwheat root samples were collected during the maturity stage using a 5-point sampling method (Carter and Gregorich, 2007). Within each plot, 5 sampling points were arranged in a quincunx pattern (4 near plot corners and 1 at the center) to ensure spatial representation while minimizing edge effects. At each point, an intact buckwheat plant was carefully excavated, and rhizosphere soil was then collected by gently shaking off the surface soil from the roots. Soil from all five points were pooled to form a composite soil sample for each plot. Upon collection, the soil samples were immediately transported to the laboratory and divided into two portions. One portion was stored at

room temperature for subsequent soil property analyses, while the other was frozen at -80°C for soil extracellular enzyme assays and DNA extraction.

For chemical analysis, soil samples were air-dried and sieved to 2 mm. Soil OM, TN, Alkali N, and TP were quantified using the dichromate oxidation method (Nelson and Sommers, 1996), the Kjeldahl method (Bremner, 1960), the steam distillation method (Chen et al., 2016), and the molybdenum blue method (Ganesh et al., 2012), respectively. TK and AK were assessed using the methods established by Tan (2005), and soil pH was measured using the conventional method (Schofield and Taylor, 1955). Acid phosphatase activity was measured using nitrophenyl phosphate disodium (PhOH mg g^{-1} , 37°C , 24 h), catalase was measured using KMnO_4 (0.1 mol L^{-1} $\text{KMnO}_4 \mu\text{g g}^{-1}$, 30°C , 20 h) (Tabatabai, 1994), and urease activity was determined using a citrate acid buffer at pH 6.7 ($\text{NH}_3\text{-N mg g}^{-1}$, 37°C , 24 h) (Kandeler and Gerber, 1988), and sucrase activity (glucose mg g^{-1}) was determined following the method described by Dick (1994).

2.2.3. Soil microbiome analysis

Soil genomic DNA was extracted using the FastDNATM SPIN Kit for Soil (Fisher Scientific, Santa Ana, CA, USA), following the manufacturer's instructions. The V4 region of ribosomal 16S rRNA gene was amplified using the primers of 551 F (5'-CCGGACTACVSGGGTATC-TAAT-3') and 806 R (5'-GTGCCAGCMGCCGCGTA-3') (Walters et al., 2016). The PCR mixture, with a final volume of 30 μL , contained 15 μL of 2 \times Phusion Master Mix (New England Biolabs, USA), 1.5 μL of each primer, 3 μL of FastPfu Polymerase (New England Biolabs, USA), and 5 μL of template DNA (1 ng μL^{-1}). The volume was adjusted to 30 μL with ddH₂O. The PCR protocol was initiated with a pre-denaturation at 98°C for 1 min, followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 50°C for 30 s, and extension at 72°C for 30 s, concluding with a final extension at 72°C for 5 min. Each sample was amplified in triplicate. The quality and quantity of PCR products were assessed using 2 % (w v⁻¹) agarose gels and a Qubit[®] 2.0 Fluorometer (Thermo Scientific) with the Agilent Bioanalyzer 2100 system, respectively. The resultant PCR products were pooled and purified using the QIAquick Gel Extraction Kit (QIAGEN, Germany). Sequencing was conducted on an Illumina NovaSeq6000 platform to produce 250 bp paired-end reads (Novogene, Beijing, China).

Raw sequencing reads were assembled using FLASH (version 1.2.7) (Magoc and Salzberg, 2011) and subjected to quality filtering in QIIME2 to retain high-quality reads with an average Phred score of ≥ 20 (Caporaso et al., 2010; Bokulich et al., 2013). Potential chimeric sequences were identified and eliminated using the UCHIME algorithm (version 4.2.40) (Edgar, 2016). Subsequently, a total of 3,059,091 reads (mean of 84,975 reads per sample) were obtained and clustered into operational taxonomic units (OTUs) at a 97 % similarity threshold using UPARSE (version 7.1) (Edgar, 2013). The yielded 10,197 bacterial OTUs were taxonomically assigned with the Silva database (Release 121) (Quast et al., 2013).

OTUs were classified into three categories based on their relative abundances, following criteria from previous studies (Jiao et al., 2017; Jiao and Lu, 2020; Zhou et al., 2022). Specifically, OTUs constituting more than 0.1 % relative abundances were classified as "abundant", whereas those comprising less than 0.01 % were categorized as "rare". OTUs with relative abundances falling between 0.01 % and 0.1 % were classified as "intermediate". Finally, we obtained 203 abundant OTUs and 8,806 rare OTUs.

2.3. Statistical analysis

To examine the effects of buckwheat species and cropping systems on diversity and community composition of bacterial abundant and rare communities, we performed the following analyses. Alpha diversity (Shannon index) was analyzed using the "diversity" function (package: vegan). For beta diversity, compositional differences among treatments

were assessed using Bray-Curtis dissimilarity, a widely used metric for ecological community data that emphasizes differences in taxon abundance while minimizing the influence of joint absences. These dissimilarities were visualized via Principal Coordinates Analysis (PCoA) using the ImageGP platform (Chen et al., 2022a). The stability of bacterial abundant and rare communities (a proxy for their resistance and resilience to environmental stress) under different cropping systems was calculated using the average variation degree method with the matrixStats function (packages: genefilter). Finally, permutational multivariate analysis of variance (PERMANOVA), a non-parametric method for testing the significance of group differences in multivariate community data, was performed to assess the effect of rotation patterns and buckwheat species on abundant and rare communities using the "adonis" function (package: vegan).

Co-occurrence networks for abundant and rare OTUs under three rotation patterns were constructed based on the Pearson correlation coefficients ($P \leq 0.05$) of log-transformed OTUs using the Molecular Ecology Network Analysis Pipeline (MENAP) following the random matrix theory (RMT) (Deng et al., 2012; Xiao et al., 2022). Only OTUs present in at least 50 % of the total samples for each group were retained for correlation calculations. RMT thresholds were set at 0.85 for both abundant and rare taxa. Topological properties, including the number of nodes and edges, average degree, clustering coefficient, path distance, geodesic efficiency, centralization (degree, betweenness, eigenvector centrality), harmonic geodesic distance, density, transitivity, connectedness, and relative modularity, were calculated to compare microbial associations and network complexity. Co-occurrence networks were visualized using Gephi with the Fruchterman-Reingold layout (Bastian et al., 2009). The robustness (reflecting the network's resistance to disturbance) of abundant and rare taxa networks under different rotation patterns was assessed through a linear model using natural connectivity as an indicator and simulating random destruction or intentional attacks by progressively removing edges (from largest to smallest weight) (Peng and Wu, 2016; Wu et al., 2021). The positive cohesion index, indicative of microbial synergistic interactions, was calculated following the approach of Herren and McMahon (2017). The complexity index for each network was calculated using nonmetric multidimensional scaling (NMDS) based on connectivity, eigenvector centrality, clustering coefficient, and the inverse of betweenness centrality, as represented by the first axis of NMDS (Zhang et al., 2024a). A more complex network suggests a diverse array of interactions, potentially leading to greater functional stability, which buffers ecosystems against disturbances. Additionally, based on within-module (Z_i) and among-module (P_i) connectivity metrics, node topological roles were classified into four categories: network hubs ($Z_i > 2.5$ and $P_i > 0.62$; highly connected across the entire network), module hubs ($Z_i > 2.5$ and $P_i \leq 0.62$; highly connected within individual modules), connectors ($Z_i \leq 2.5$ and $P_i > 0.62$; nodes linking different modules), and peripherals ($Z_i \leq 2.5$ and $P_i < 0.62$; nodes with limited connections, mostly within their own modules). Functional predictions for these nodes were made using the FAPROTAX database (Louca et al., 2016).

A random forest model was used to identify abundant and rare bacterial taxa that differentiated between samples from the three rotation patterns using the "randomForest" function (package: randomForest). Ten-fold cross-validation with five repeats was applied to evaluate the importance of bacterial OTUs using the Mean Decrease Gini values (Fig. S2). A bubble map was generated to visualize the normalized relative abundances of biomarker OTUs across treatments. Pearson's correlations between the relative abundance of these biomarkers and yield were calculated using the "rcorr" function (package: Hmisc).

PICRUSt2 was employed to predict the potential functions of abundant and rare taxa (Douglas et al., 2020). The bioindicators of predicted functions across three rotation patterns were assessed using a linear discriminant analysis of effect size (LefSe) following the method of Segata et al. (2011). The Pearson correlation analysis was performed to test the relationships between the bioindicators of predicted functions

and yield using the “cor” function (package: corplot), and *P* values were adjusted for multiple testing with the Bonferroni-Holm method.

Partial least squares path modeling (PLS-PM) was conducted to assess relationships among seven latent variables (buckwheat species, rotation, soil chemical properties, soil enzyme activities, abundant bacterial taxa, rare bacterial taxa, and buckwheat yield) using the “plspm” function (package: plspm) (Hair et al., 2021). Buckwheat species variable was built by assigning the value 1 to common tartary and 0 to tartary buckwheat, and rotation patterns were created by assigning the value 2 to the soybean-buckwheat rotation, 1 to the wheat-buckwheat rotation, 0 for continuous mono-buckwheat. A set of 22 manifest variables contributing to these latent variables were selected based on correlation analysis results, including soil chemical properties (TP, pH, TK, TN, OM, AK, Alkali N), enzymes (acid phosphatase, catalase, sucrase, urease), abundant and rare bacterial communities (observed OTUs, composition as indicated by first PcoA score, network complexity, and bioindicators of predicted functions). Before model construction, all variables were standardized using Z-transformation using the “scale” function. The optimal model was determined with the goodness of fit index (*GoF* > 0.50) and the highest determination coefficients (*R*² > 0.60).

Significant differences in key network topological properties (cohesion and complexity indices) and predicted metabolic functions of abundant and rare taxa among different rotation types were determined using a one-way ANOVA. A two-way ANOVA was used to examine the effect of rotation systems and buckwheat species on soil chemical properties, enzyme activities, relative abundance of top 10 bacterial abundant and rare taxa at the phylum level, alpha diversity and stability of abundant and rare communities. A three-way ANOVA was used to assess the effect of rotation systems, buckwheat species, and sampling year on yield and yield-related parameters. Tukey’s HSD test at the 5% significance level was applied for post hoc comparisons to identify significant differences among treatments. Graphs were created using GraphPad Prism 9.5, with data presented as mean ± standard error for each parameter.

3. Results

3.1. Yields of different buckwheat species under three cropping patterns

There was a significant interaction of sampling year and rotation systems in buckwheat yield (Fig. 1B). Over the period from 2018 to

2020, average yields in the R1 and R3 patterns increased by 9.39 % and 14.57 %, respectively, while the R2 pattern experienced a decrease of 9.98 %. The highest yield of buckwheat (1180.4 kg ha⁻¹) was recorded in the R3 pattern in 2020, whereas the lowest yield (880.6 kg ha⁻¹) was in the R2 pattern during the same year. Across all rotation systems from 2018 to 2020, common buckwheat yields (1054.5, 1078.9, and 1085.8 kg ha⁻¹) were significantly higher (*P* < 0.001) than those of tartary buckwheat (943.5, 974.4, and 1009.0 kg ha⁻¹). However, tartary buckwheat displayed more yield improvements under rotations, with increases of 18.76 % in R3 (ST) and 11.13 % in R1 (WT), as opposed to increases of 10.84 % (SC) and 7.87 % (WC) for common buckwheat in the same patterns, respectively. Other yield parameters, including 1000-grain weight, grain number, and grain weight per plant, showed the similar trends observed in average yield (Table S1).

3.2. Effects of cropping patterns and buckwheat species on soil properties

Our two-way ANOVA results indicated no significant interactions between rotation patterns and buckwheat species in soil-related properties (*P* > 0.05; Table 1). However, rotation pattern was the main effect that significantly influenced most soil properties, including pH (*P* < 0.001), OM (*P* < 0.001), TN (*P* < 0.001), Alkali N (*P* < 0.05), TK (*P* < 0.05), TP (*P* < 0.001), catalase (*P* < 0.001), sucrase (*P* < 0.001), and acid phosphatase (*P* < 0.001; Table 1). Post-hoc Tukey’s HSD tests showed that soil pH, TN, and Alkali N were significantly higher in R1 relative to R2 and R3. Soil OM were significantly greater in R3 and R1 than in R2. TK had a significantly higher value in R1 and R2, while TP levels were significantly greater in R2 and R3. Soil enzyme activities, including catalase, sucrase, and acid phosphatase, were highest in R1, followed by R2 and R3. Buckwheat species had a significant impact on soil AK content (ANOVA; *P* < 0.05), with tartary buckwheat (286.7 mg kg⁻¹) exhibiting 68.08 % higher than common buckwheat (170.6 mg kg⁻¹). In contrast, soil urease activity was unaffected by either rotation pattern or buckwheat species (ANOVA; *P* > 0.05).

3.3. Differences in abundant and rare bacterial communities across three cropping patterns

Rotation systems exerted a significant influence on the relative abundance of both dominant bacterial abundant and rare taxa (Top 10; *P* < 0.001) at the phylum level, with the exception of abundant Cyanobacteria and rare Proteobacteria and Armatimonadetes (Fig. 2A;

Table 1

Effects of rotation systems and buckwheat species on soil properties based on a two-way ANOVA.

Soil properties	R1		R2		R3		ANOVA
	WC	WT	BC	BT	SC	ST	
pH	5.9 ± 0.1a	5.8 ± 0.1a	5.4 ± 0.1ab	5.3 ± 0.1b	5.4 ± 0.1ab	5.5 ± 0.1 ab	Rotation* **
OM (g kg ⁻¹)	9.5 ± 0.2a	9.7 ± 0.1a	5.4 ± 0.2b	5.4 ± 0.2 b	9.8 ± 0.4a	10.3 ± 0.3a	Rotation* **
TN (%)	13.2 ± 0.6ab	13.6 ± 0.2a	10.0 ± 1.3c	9.2 ± 0.4c	10.5 ± 0.3bc	10.8 ± 0.9bc	Rotation* **
Alkali-N (mg kg ⁻¹)	10.2 ± 0.4ab	11.6 ± 0.9a	7.2 ± 0.3b	9.4 ± 1.4 ab	8.7 ± 0.5ab	8.5 ± 0.5ab	Rotation* *
TK (%)	27.4 ± 1.1	28.0 ± 0.8	27.2 ± 0.3	27.6 ± 0.6	26.3 ± 1.4	24.5 ± 1.0	Rotation*
AK (mg kg ⁻¹)	236.8 ± 41.4	299.6 ± 42.7	229.3 ± 40.4	301.8 ± 41.2	216.3 ± 25.9	258.8 ± 7.8	Species*
TP (%)	7.0 ± 0.3b	7.3 ± 0.4b	9.1 ± 0.1a	8.9 ± 0.2a	9.4 ± 0.2 a	9.0 ± 0.4 a	Rotation* **
Urease (NH ₃ -N mg g ⁻¹)	0.5 ± 0.02	0.4 ± 0.03	0.5 ± 0.03	0.5 ± 0.02	0.5 ± 0.01	0.5 ± 0.01	N.S.
Catalase (KMnO ₄ µg g ⁻¹)	0.6 ± 0.02a	0.6 ± 0.03ab	0.5 ± 0.04ab	0.5 ± 0.03b	0.3 ± 0.02c	0.4 ± 0.04c	Rotation* **
Sucrase (glucose mg g ⁻¹)	0.5 ± 0.04a	0.4 ± 0.02 ab	0.5 ± 0.04 a	0.4 ± 0.06 ab	0.4 ± 0.03ab	0.3 ± 0.01b	Rotation* **
Acid phosphatase (PhOH mg g ⁻¹)	172.3 ± 7.2a	180.8 ± 0.7a	174.0 ± 5.7a	163.7 ± 8.0ab	147.0 ± 2.6b	145.8 ± 2.1b	Rotation* **

Values are presented as mean (n = 6) ± standard error. Significant differences among six treatments were determined using the Tukey’s HSD test, denoted by lowercase letters. * **, * *, and * represent the significance at *P* < 0.001, 0.01, and 0.05 respectively. N.S. indicates no significant effects. OM: organic matter; TN: total nitrogen; TP: total phosphorus; TK: total potassium; Alkali N: alkali nitrogen; AK: available potassium.

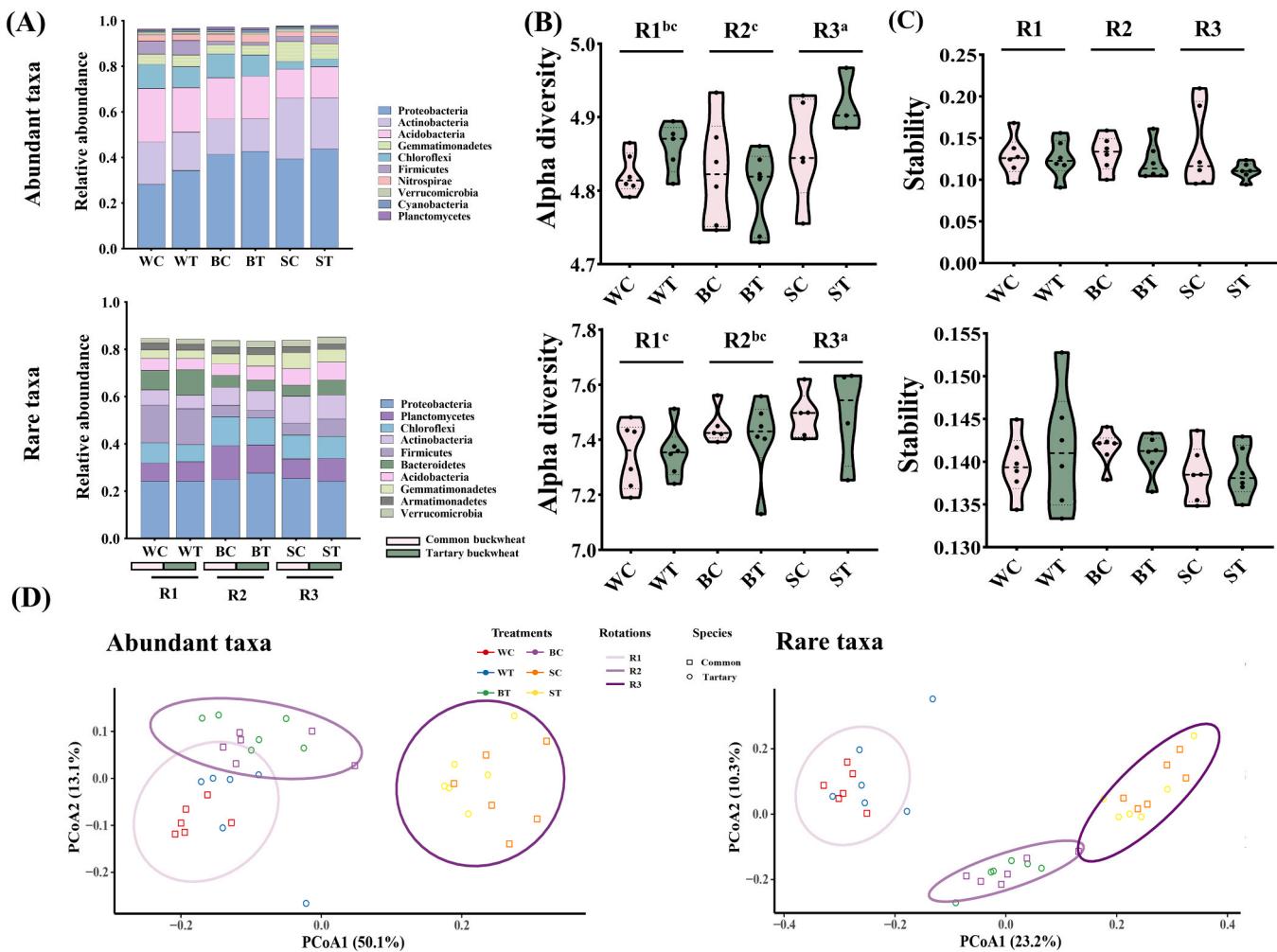


Fig. 2. Variations in abundant and rare bacterial communities within the buckwheat rhizosphere across different rotation systems and buckwheat species. (A) The relative abundance of the top 10 bacterial phyla of abundant and rare communities. (B) Alpha diversity measured by the Shannon Index. Lowercase letters indicate significant differences among rotation systems based on a Tukey's HSD test when the rotation system was the main effect. (C) Stability index of soil bacterial communities under different rotation systems and buckwheat species. (D) Beta diversity calculated with a PCoA of Bray–Curtis distance among treatments.

Table S2). Proteobacteria were the most prevalent across all treatments, constituting a larger proportion of abundant (38.32 %) and rare taxa (21.14 %). Among abundant taxa, Proteobacteria, Actinobacteria, and Acidobacteria collectively accounted for 75 % of the community, while other phyla such as Chloroflexi (7.76 %), Gemmatimonadetes (5.54 %), and Firmicutes (3.39 %) were present in smaller proportions. In contrast, rare taxa, except Proteobacteria, exhibited a more even distribution, with mean relative abundances ranging from 2 % to 10 % across treatments.

We observed significant effects of rotation patterns on the alpha diversity (Shannon index) of both abundant and rare communities, with R3 displaying the largest values for both community types, regardless of buckwheat species (Fig. 2B; Table S3). However, community stability of both abundant and rare bacterial communities remained unaffected by either rotation systems or buckwheat species (Fig. 2C; Table S3). Further, our PERMANOVA analysis combined with PCoA plots of Bray–Curtis distances showed that rotation patterns significantly impacted the community structure of both abundant and rare taxa ($P < 0.001$), with no effect of buckwheat species (Fig. 2D; Table 2). Notably, rotation patterns had stronger effects on abundant communities ($R^2 = 0.55$) compared to rare communities ($R^2 = 0.31$) (Table 2).

Table 2

Effects of rotation patterns and buckwheat species on community composition of bacterial abundant and rare taxa based on PERMANOVA.

	Species	Rotations	Species × Rotations
Abundant taxa	R^2	0.02	0.04
	F	1.64	20.54
	P	0.15	0.001 ***
Rare taxa	R^2	0.02	0.04
	F	0.84	7.23
	P	0.62	0.001 ***

3.4. Co-occurrence network of abundant and rare taxa under different rotation patterns

Given that buckwheat species had limited effects on both abundant and rare communities (Fig. 2; Table 2; Table S2), co-occurrence networks were constructed based on Spearman correlations among OTUs of abundant and rare taxa to investigate microbial inter-connections under different rotation patterns (Fig. 3A). Rotation patterns strongly influenced network topological properties of both abundant and rare microbial taxa (Table 3). Specifically, compared to the continuous buckwheat rotation pattern (R2: abundant - 110 links, 86 nodes; rare - 1444 links, 1227 nodes), the more functional diversified rotation R3

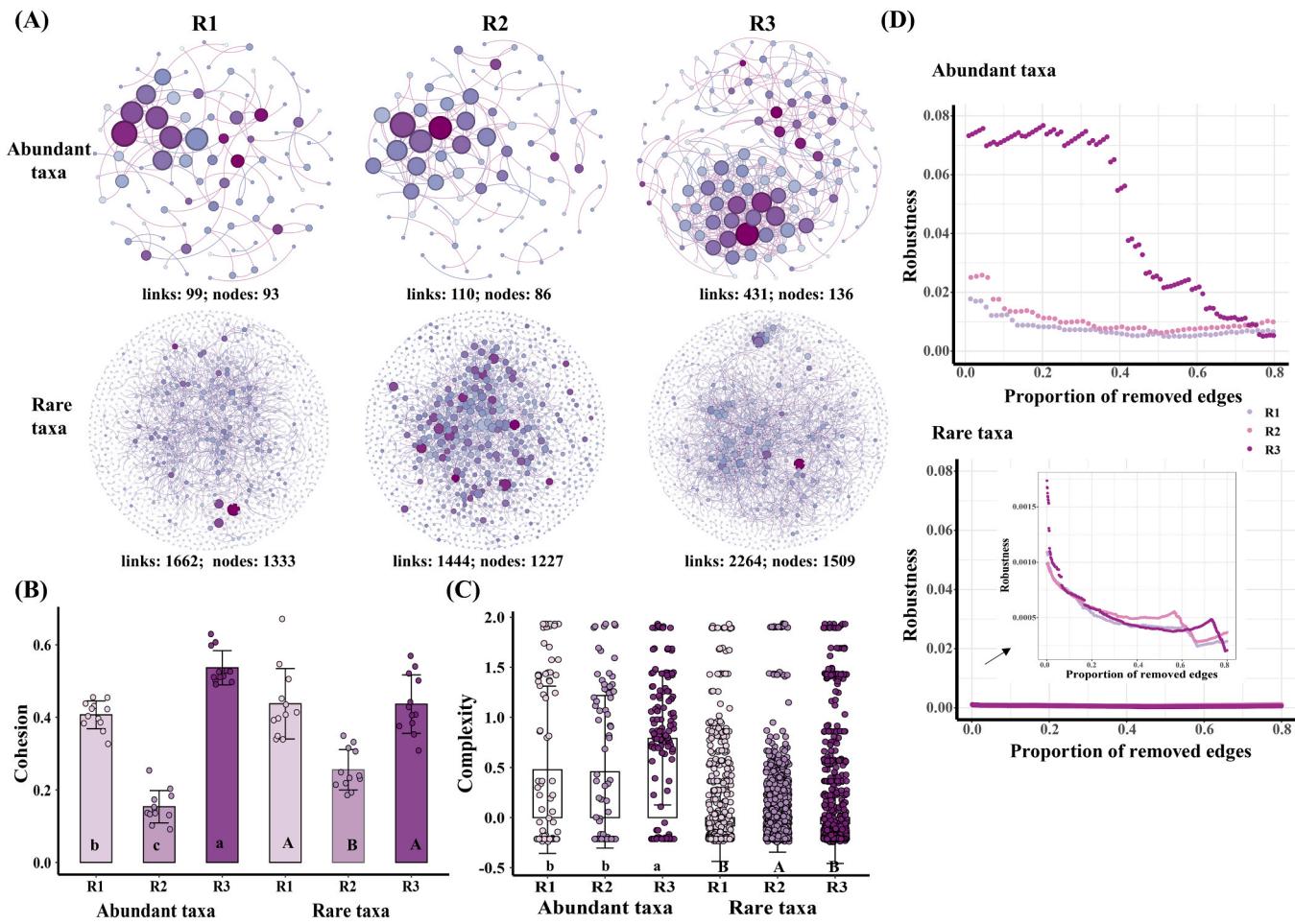


Fig. 3. Co-occurrence patterns of soil bacterial abundant and rare taxa across three rotation systems (A). Each node represents a bacterial OTU, with the node size proportional to its relative abundance. Node color (purple-blue and gray) corresponds to high and low Zi values, respectively. The pink and blue edges represent positive and negative correlations, respectively. Comparison of network key topological properties, including positive cohesion index (B), complexity index (C), and robustness (D). Different lowercase and uppercase letters on columns indicate significant differences in abundant and rare taxa across rotations ($P < 0.05$), respectively, determined by a Tukey's HSD test. Specific topological properties of bacterial abundant and rare co-occurrence patterns at rotation systems are shown in Table 3.

Table 3

Key topological properties of co-occurrence networks of abundant and rare bacterial taxa under different rotation patterns.

Network characterizations	Abundant taxa			Rare taxa		
	R1	R2	R3	R1	R2	R3
R^2 of power-law	0.83	0.87	0.81	0.92	0.86	0.92
Average degree	2.13	2.56	6.34	2.49	2.35	3.00
Average clustering coefficient	0.20	0.16	0.30	0.02	0.02	0.03
Average path distance	4.00	3.85	4.89	7.64	7.58	7.00
Geodesic efficiency	0.37	0.37	0.30	0.15	0.15	0.16
Centralization of degree	0.07	0.09	0.16	0.02	0.01	0.02
Harmonic geodesic distance	2.71	2.70	3.36	6.74	6.63	6.26
Centralization of betweenness	0.08	0.13	0.11	0.004	0	0.02
Centralization of eigenvector centrality	0.35	0.34	0.25	0.48	0.36	0.39
Density	0.02	0.03	0.05	0.002	0.002	0.002
Transitivity	0.35	0.28	0.31	0.02	0.01	0.03
Connectedness	0.17	0.27	0.79	0.59	0.50	0.67
Modularity	0.78	0.64	0.49	0.84	0.83	0.78
Positive correlations	82.8 %	77.3 %	71.9 %	28.6 %	26.5 %	34.9 %

R^2 values of the power-law indicate the presence of scale-free networks.

(abundant - 431 links, 136 nodes; rare - 2264 links, 1509 nodes) had a greater number of links and nodes in networks of both abundant and rare taxa, leading to greater network average degrees (Fig. 3A, Table 3). Rotation systems also significantly impacted the positive cohesion (Fig. 3B) and complexity (Fig. 3C) indices for networks of both abundant

and rare communities. Specifically, R3 exhibited significantly higher positive cohesion and complexity in abundant taxa network compared to R2 and R1 (Fig. 3B-C). In the rare taxa network, R1 and R3 had significantly greater cohesion relative to R2, whereas complexity showed the opposite trend. In contrast, effects of rotation patterns on

robustness (measured by natural connectivity values after randomly removing 60 % of the network edges; Fig. 3D) were observed only in abundant taxa, where R3 had a higher level of robustness compared to R1 and R2.

3.5. Biomarkers of abundant and rare taxa under different rotation patterns

Random forest models were employed to identify key biomarkers of abundant and rare taxa at the OTU level across different rotation patterns. We identified the most important (top 14) abundant and rare OTUs, based on Mean Decrease Gini values (Fig. 4A; Table S4). Among the abundant OTU biomarkers, seven, including OTU 67 (Chloroflexi), OTU 2959 (Proteobacteria), OTU 565 (Gemmatimonadetes), OTU115 (Gemmatimonadetes), OTU 82 (Actinobacteria), OTU 151 (Gemmatimonadetes), OTU 57 (Gemmatimonadetes), showed higher relative abundances in R3, and five - OTU 11 (Gemmatimonadetes), OTU 87 (Actinobacteria), OTU 126 (Chloroflexi), OTU 148 (Firmicutes), OTU 86 (Chloroflexi) - had higher relative abundances in R1. In contrast, for the rare OTU biomarkers, most of them, including OTU 1117 (unidentified Phylum), OTU 1074 (Bacteroidetes), and OTU 1041 (Firmicutes), OTU 1069 (Firmicutes), OTU 1671 (Firmicutes), OTU 2493 (Chloroflexi), OTU 1089 (Chloroflexi), OTU 1721 (Actinobacteria), were more abundant in R1, while only two OTUs, i.e., OTU 1358 (Acidobacteria) and OTU 1236 (Actinobacteria), displayed higher relative abundances in R3. Together, the identified biomarkers were predominantly from the phyla Chloroflexi (28.57 %), Gemmatimonadetes (17.86 %), Actinobacteria (14.29 %), and Proteobacteria (14.29 %) (Fig. 4A).

Further Pearson's correlation analysis showed that six abundant

OTU biomarkers, including OTU 67, OTU 2959, OTU 565, OTU 115, OTU 82, and OTU115 which mainly belonged to the phyla of Gemmatimonadetes, Proteobacteria, Actinobacteria, and Chloroflexi were significantly and positively correlated with buckwheat yields (Fig. 4 B). Conversely, rare OTU biomarkers - OTU 1989 (Planctomycetes) and OTU 1814 (Proteobacteria) - showed negative correlations with buckwheat yield (Fig. 4 B).

3.6. Functional prediction of abundant and rare bacterial communities across three rotation patterns

To elucidate the functional differences of soil abundant and rare communities under three different crop rotations, we employed PICRUSt2 for predictive functional profiling. We identified six primary KEGG pathway classifications, predominantly categorized under metabolic functions, which accounted for 74.64 % and 73.53 % of relative abundance in abundant and rare taxa, respectively (Fig. 5A). To further analyze these variances in dominant metabolic functions across the rotation patterns, the LefSe method was subsequently employed (Fig. 5B; Table S5). Our findings showed that a greater number of functional pathways were enriched in R3 and R1 than in R2 for both abundant and rare communities. Specifically, in R3, pathways associated with xenobiotics biodegradation and metabolism, terpenoids and polyketides metabolism, and amino acid metabolism were significantly enriched in both abundant and rare taxa, in addition to energy metabolism that was only enriched in abundant taxa. The R1 rotation also showed significant pathway enrichment, with glycan biosynthesis and metabolism, and carbohydrate metabolism being abundant in both abundant and rare taxa. Additionally, biosynthesis of other secondary

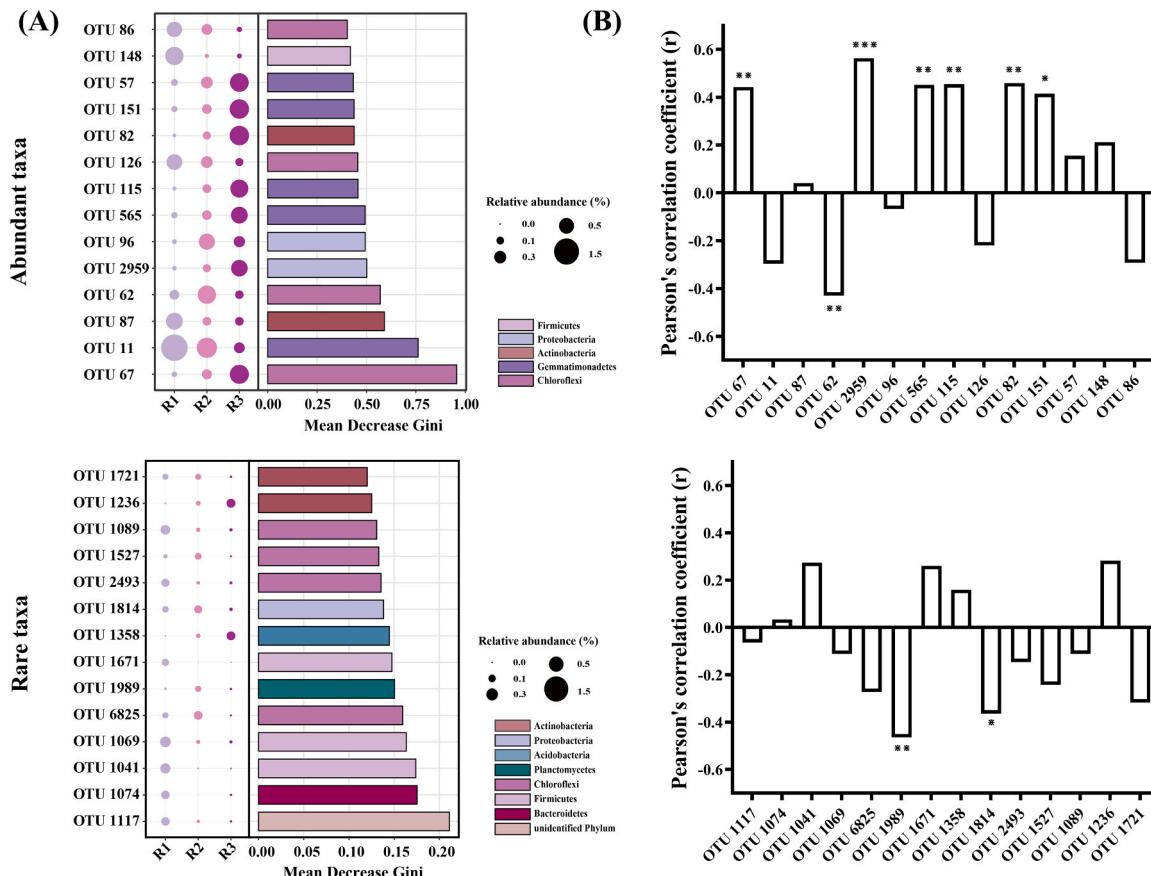


Fig. 4. Abundant and rare biomarkers predicted by the Random Forest model under different rotation patterns (A). The top 14 abundant and rare OTUs were determined with the mean decrease Gini. Model performance (the smallest out-of-bag estimated relative error rates and bacterial taxa cross-validation error rates) is shown in Fig. S2A. Pearson correlations between biomarkers and buckwheat yields (B). Asterisks indicate statistical significance (**: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$).

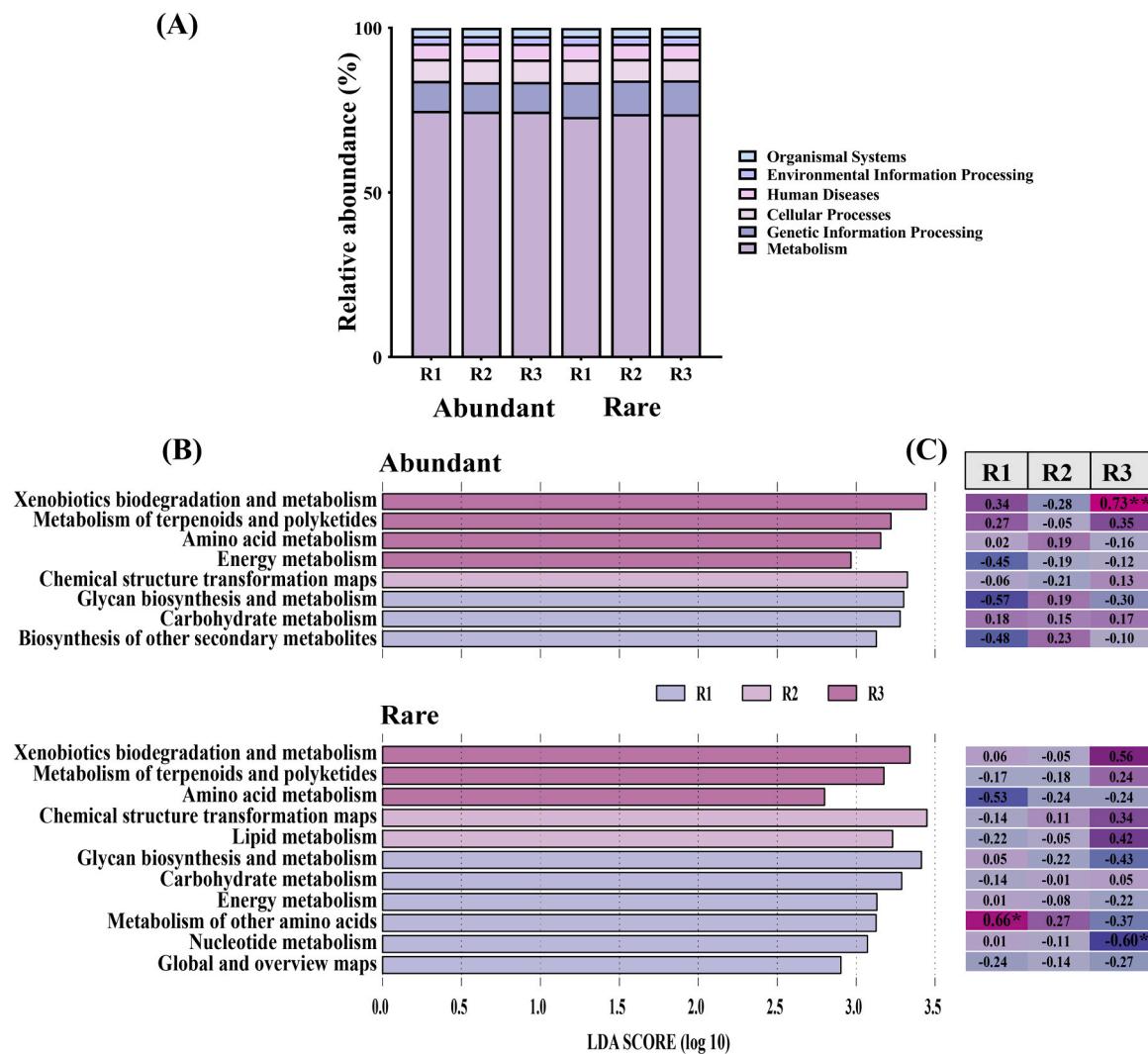


Fig. 5. Variations in the predicted functions of abundant and rare communities across three rotation systems (A). LefSe analysis detecting the bioindicators of predicted functions across three rotation patterns (B). Heatmap of correlations between the bioindicators of predicted functions and buckwheat yields (C). * and ** indicate significance at $P < 0.05$, and 0.01 , respectively, determined by the Pearson correlation coefficient after correction for multiple comparisons using the Bonferroni-Holm method.

metabolites was particularly enriched in the abundant taxa, while energy metabolism, metabolism of other amino acids, nucleotide metabolism, and global and overview maps were prevalent in rare taxa. In contrast, the R2 rotation had limited enrichment, confined to pathways related to chemical structure transformation in abundant taxa and chemical structure transformation and lipid metabolism pathways in rare taxa.

To elucidate the connection between bioindicators of predicted functions and yield, Pearson correlation analysis was conducted (Fig. 5C). Interestingly, significant correlations were observed only in the R1 and R3 rotation patterns, with no relationships detected in R2. Specifically, xenobiotics biodegradation and metabolism associated with abundant taxa in R3 had a positive correlation with buckwheat yield. In contrast, within the rare taxa, metabolism of other amino acids was positively correlated with buckwheat yield in R1, but nucleotide metabolism was negatively linked to yield in R3.

3.7. Linkages among buckwheat species, rotation patterns, soil properties, and microbial communities, and buckwheat yield

A Partial Least Squares Path Modeling (PLS-PM) analysis was conducted to delineate the interrelationships among buckwheat species,

rotation patterns, soil abundant and rare bacterial communities, soil enzyme activities, soil chemical properties, and buckwheat yield (Fig. 6). The analysis revealed that diversified rotation patterns significantly enhanced the attributes of both abundant (path coefficient = 0.74, $P < 0.001$) and rare (path coefficient = 0.66, $P < 0.001$) bacterial communities. Furthermore, rare taxa were negatively associated with soil enzyme activities (path coefficient = -0.81, $P < 0.001$), which, in turn, positively affected soil chemical properties (path coefficient = 0.54, $P < 0.05$). Conversely, abundant taxa played a crucial role in enhancing buckwheat yield (path coefficient = 0.92, $P < 0.001$).

4. Discussions

4.1. Rotation systems, not buckwheat species, structured rhizosphere bacterial communities

Our study demonstrated that rotation systems exerted a significantly greater influence on the diversity and composition of both abundant and rare microbial communities in the rhizosphere compared to buckwheat species (Fig. 2; Table 2). This is consistent with findings from a comprehensive study across 114 typical dryland farmlands in northeastern China, which showed that crop rotation systems enhanced the

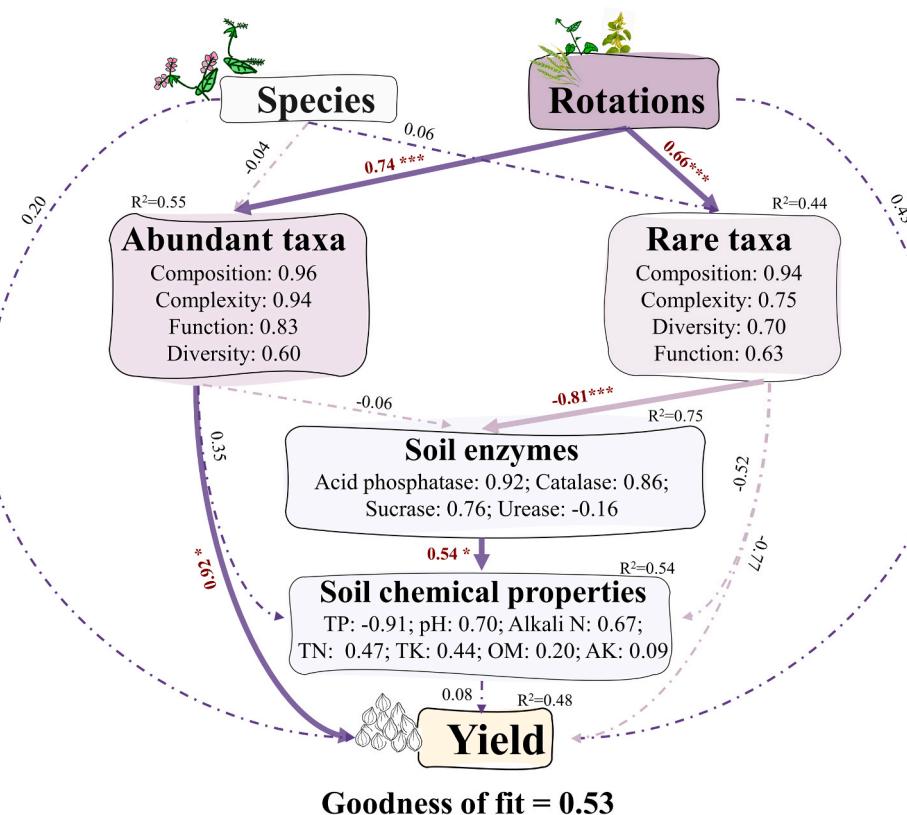


Fig. 6. Partial least squares path modeling illustrating interrelationships among buckwheat species, rotation patterns, attributes of abundant and rare bacterial communities, soil enzymes, soil chemical properties, and buckwheat yields. Path coefficients illustrate the direction and magnitude of relationships between variables, while the coefficients of determination (R^2) indicate the proportion of variance in latent variables explained by the manifest variables included in their calculation; both metrics were derived using 1000 bootstraps. Latent variables are highlighted in bold within each box and are inferred from manifest variables, with loading values next to each manifest variable illustrating the strength of its relationship to the corresponding latent variable. Solid and dashed arrows indicate the significant and non-significant relationships, respectively, and dark purple and pink colors indicate the positive and negative relationships, respectively. Path coefficients are displayed adjacent to the arrows, and the width of arrows shows the strength of path coefficients. *, **, and *** indicate significance at $P < 0.05$, 0.01 , and 0.001 , respectively. TP: total phosphorus; TK: total potassium; TN: total nitrogen; OM: organic matter; Alkali N: alkali nitrogen; AK: available potassium.

alpha diversity and structural composition of both abundant and rare sub-communities (Zhou et al., 2022). Notably, integrating leguminous crops (i.e., soybean) with buckwheat significantly boosted alpha diversity in both abundant and rare bacterial communities compared to the buckwheat monoculture. Crop diversification can significantly enrich the rhizosphere, improving the quantity, quality, and chemical diversity of C inputs primarily through rhizodeposition, including a variety of compounds such as carbohydrates, organic and amino acids, phenolics, fatty acids, sterols, enzymes, vitamins, hormones, and nucleosides (Jones et al., 2009; Zhang et al., 2021). This enrichment can ultimately contribute to soil C accrual (as reflected by increased soil OM) (Pribyl, 2010) and nutrient availability (Table 1). Importantly, these benefits extended beyond the immediate cropping cycle. In our study, such effects appeared to confer a legacy benefits to subsequent crops such as buckwheat, attracting a greater diversity of microbes from the bulk soil into the rhizosphere (Fig. 2B), altering rhizosphere microbial communities and fostering a more resilient soil ecosystem (Zhang et al., 2021).

4.2. Mechanisms underlying the contribution of abundant taxa to crop yield

Interestingly, our PLS-PM suggested that abundant and rare microbial communities regulated by rotation systems played divergent roles in agroecosystem functioning (Fig. 6). Specifically, the attributes of abundant taxa, particularly enhanced by diversified crop rotations, significantly contributed to buckwheat yield. Co-occurrence network

analysis indicated that the diversified rotation system positively affected key topological properties of the abundant microbial co-occurrence network, such as cohesion, complexity, and robustness (Fig. 3), which in turn significantly impacted buckwheat yield (Fig. 6). The enhanced positive cohesion, complexity, and robustness observed in R3 suggest that the diversified rotation system may foster more stable and functionally interconnected microbial networks (Hernandez et al., 2021). These attributes likely supported complementary functions among abundant communities and increased the overall resilience of rhizosphere microbiomes to abiotic and biotic stresses commonly encountered in mountainous agroecosystems (Chen et al., 2022b). In practice, these features can contribute to more consistent belowground ecosystem functioning, ultimately promoting greater crop productivity and agroecosystem sustainability. This is supported by the observed reduction in catalase activity, indicative of lower baseline oxidative stress (Stpniekska et al., 2009), and enhanced pathways associated with xenobiotics biodegradation and metabolism, terpenoids and polyketides metabolism, amino acid metabolism, and energy metabolism in R3 relative to R2. Furthermore, we found that a positive correlation between buckwheat yield and functions associated with xenobiotic biodegradation and metabolism (Fig. 5C). Xenobiotics, typically originating from synthetic pesticides and fertilizers, can pose toxicity risks to crops due to their persistence and minimal biodegradation in soil (Katayama et al., 2010). However, certain taxa from the phyla Proteobacteria, Actinobacteria, and Firmicutes are known to co-utilize these compounds as substrates, facilitating their degradation (Regar et al., 2019; Jokhakar et al., 2022). This functional potential may help mitigate

the toxic effects of xenobiotics on agroecosystems, promote soil health, and ultimately enhance crop productivity (Rebello et al., 2021). However, the underlying mechanisms driving this process (Chandrasekaran and Paramasivan, 2024) under the diversified rotation systems warrant further investigation in future studies.

Furthermore, a majority of abundant biomarkers detected, particularly those with the highest relative abundance in R3 (OTU 67, OTU 2959, OTU 565, OTU 115, OTU 82, and OTU 151), displayed positive correlations with buckwheat yield (Fig. 4). This is consistent with the study of Ma et al. (2023), who demonstrated that bacterial biomarkers predict crop yield more effectively than overall bacterial community assessments. Biomarkers are crucial for indicating soil health, potential agricultural productivity, and the environmental impact of agricultural practices, all of which may enhance crop growth (Soman et al., 2017). For example, some studies have identified the positive association between the relative abundance of *Gemmaitimonas* (OTU 151) and the yields of crops such as soybean and cabbage (Wang et al., 2024a; Zhang et al., 2024b), highlighting its growth-promoting capability. However, the specific mechanisms through which *Gemmaitimonas* influences plant growth are still not well understood, and the processes through which diversified rotation systems selectively enriched these biomarkers need to be further explored.

4.3. Rare taxa mediated soil chemical properties by regulating enzymatic activities

In contrast, rare taxa significantly influenced soil chemical properties by modulating soil enzyme activities. Notably, we found that crop rotation systems, particularly the diversified rotation system R3, exhibited a greater number of both keystone nodes (connectors, module hubs, and network hubs) and peripheral nodes in rare networks compared to the monoculture system R2 (Fig. 3A; Table S6). While keystone nodes are widely recognized for maintaining network connectivity and stability (Yuan et al., 2021; Zhang et al., 2022b), peripheral nodes, albeit less connected, can exert distinct and meaningful ecological functions in agroecosystems. For example, peripheral nodes, especially in R3, were far more numerous than keystone nodes, suggesting greater taxonomic richness and the potential to act as reservoirs of functional diversity (Tables S6-9). This diversity may support fine-scale regulation of critical nutrient transformations, such as C, N, and sulfur cycling, micronutrient solubilization (e.g., manganese), and the degradation of specific organic compounds such as aromatic hydrocarbon (Table S9). Importantly, several unique microbial functions related to nutrient cycling, such as chitinolysis, xylanolysis, ligninolysis, and iron respiration, were uniquely enriched in R3 and largely absent in R2. These findings partially align with previous research indicating that rare bacterial taxa are essential drivers of soil multi-nutrient cycling and ecosystem functioning across various agricultural management systems (Lynch and Neufeld, 2015). Interestingly, our results also indicated that the diversified rotation system might reduce the activity of certain soil enzymes (acid phosphatase, catalase, and sucrase). This reduction can be attributed to the combined legacy and rhizosphere effects induced by this practice, which increased overall nutrient availability. As a result, microbial communities may shift toward more specialized or diverse enzymatic pathways, decreasing the reliance on dominant enzyme activities (Table S7-9). This hypothesis was supported by our observations of elevated levels of key soil properties (i.e., OM, TN, and Alkali N) in R1 and R3 compared to R2 (Table 1). In other words, monocropping appeared to deplete soil nutrients, as continuous cultivation of the same crop buckwheat resulted in the repetitive extraction of similar nutrients, ultimately reducing soil fertility over time (Alemew and Alemayehu, 2020). Overall, these findings highlight the complex ecological dynamics facilitated by rare taxa under the diversified rotation system and their essential role in maintaining agroecosystem multifunctionality, with the potential to reduce reliance on synthetic inputs (Jousset et al., 2017; Xue et al., 2020; Xiong et al., 2021; Zhou et al., 2022).

5. Conclusion

By characterizing abundant and rare microbial communities in the buckwheat rhizosphere across different cropping systems and buckwheat species, this study advances our understanding of the interrelationships among rhizosphere microbiomes, soil functioning, and crop productivity. We found that diversified rotation patterns, particularly the diversified soybean-buckwheat rotation, had a stronger influence on rhizosphere microbial communities than buckwheat species, enhancing agroecosystem functioning through distinct contributions from abundant and rare taxa. Specifically, abundant taxa, strengthened by rotational diversity, enhanced crop yield by fostering greater network complexity, synergistic interactions, and robustness, as well as assembling more yield-associated biomarkers. In contrast, rare taxa primarily regulated soil chemical properties by modulating nutrient cycling. Overall, this study highlights the importance of high rotational diversity in intensifying ecological connections among soil, microbes, and plants, and delineates the distinct roles that abundant and rare microbial communities play in agroecosystem functioning. Therefore, harnessing the functional potential of rhizosphere microbial communities, particularly through microbiome-informed rotation strategies that incorporate legumes, offers a promising pathway toward building more resilient, productive, and environmentally sustainable agricultural systems. However, further research is needed to track microbial succession to validate these findings in long-term field trials and uncover the molecular mechanisms that underlie the functional contributions of these microbial groups.

CRediT authorship contribution statement

Zelin Yi: Supervision, Project administration, Funding acquisition, Conceptualization. **Mengqi Ding:** Writing – review & editing, Project administration, Data curation. **Kaile Zhang:** Writing – review & editing, Data curation. **Zhuo Liu:** Formal analysis, Data curation. **Yuanhao Yang:** Formal analysis, Data curation. **Xiaomei Fang:** Visualization, Supervision, Funding acquisition, Conceptualization. **Zhen Wang:** Validation, Formal analysis. **Huan Luo:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Zhiyong Zhang:** Validation, Methodology, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2025.109781.

Data Availability

Data will be made available on request.

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