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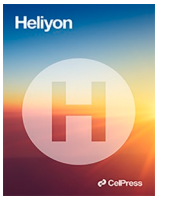

Research article

# The choice of rice rotation system affects the composition of the soil fungal community and functional traits

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ABSTRACT

Plant rotation is a common practice in upland rice production. However, the effects of plant rotation on the interactions between rice plants, soil and underground ecosystems need to be studied further. Here, quantitative PCR and high-throughput pyrosequencing of the ITS region was applied to investigate the fungal abundance, diversity, and composition of fungal functional guilds in rice field soils and after different rotation practices ((rice-fallow (RF), rice-Chinese milk vetch (RV) and rice-wheat (RW)) and their relationship with rice yields. The results showed that the six-year RV and RW rotations increased fungal abundance by 42.7 %–69.2 % relative to RF, but decreased the soil bacterial-to-fungi ratio and fungal diversity. For the functional guilds, RV rotation significantly increased the relative abundance of soil saprotrophs and pathotrophs by 73.30 % and 32.94 %, respectively, while that of symbiotrophs was decreased by 35.96 %, compared to RF. RW rotation was found to significantly decrease all three fungal functional guilds, but increased the symbiotroph-saprotroph ratio. A structure equal model analysis indicated that the diversity of saprotrophs was significantly and negatively correlated with rice yield. Altogether, this work provides a detailed description of how the soil fungal community, including saprotrophic, symbiotrophic and pathotrophic functional guilds, responded to different upland rice rotation practices after eight years of application.

## 1. Introduction

Rice (*Oryza sativa* L.) is cultivated all over the word and provides the daily diets for more than half of the world's human population. It is reported that China and India alone produce and consume about 50 % of the global rice production [1]. It was reported that around 510 million metric tons of rice were produced annually in the world [2], and a higher production of higher quality rice will become crucial for global food security and the maintenance of human living standards.

The main determinants of rice yields include soil fertility, management practices, climate conditions and the rice genetic variety utilized [3,4]. Crop rotation is also a critical practice to determine rice yield. For example, rice-Chinese milk vetch rotation can increase rice yield by 14.6 % in a red paddy soil [5]. In addition, upland rice rotation could also reduce the global warming potential,

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decrease greenhouse gas emissions, and increase nitrogen use efficiency and soil fertility [6–8].

Soil fungi, which exerts vital functions in soil health, carbon sequestration and crop yield, are comprised of distinct ecological guilds [9]. Saprotrophic fungi can decompose soil organic matter, contributing to carbon cycling and promoting soil structure formation [10]. Symbiotrophic fungi, which form symbiotic relationships with many land plants, can facilitate plant access to nutrients and water in exchange for carbon [11]. Pathotrophic fungi include those that can cause plant disease but some may be beneficial in agriculture through their control of plant, animal, or fungal pests [12,13]. A higher soil fungal diversity and abundance can increase ecosystem stability and productivity [14], which may be possible by the reduction of soil pollutants via a bioremediation process [15,16]. It has been shown that upland rice rotation affects the soil microbial community composition and diversity. For example, Zhang et al. (2017) [17] found that a 31-year period of upland rice rotation increased soil microbial abundance but decreased its α-diversity, with a shift in the microbial composition to those with plant growth promoting traits. However, the effects of upland rice rotation on rice field soil fungal compositions remain unclear.

To better understand how different upland rice rotation practices impact the fungal diversity and composition, a field experiment in 2014 where rice was rotated with a fallow season (RF), Chinese milk vetch (RV) or wheat (RW) was established, which are the three major rice rotation systems currently used in the Yangtze Delta of China. The main objectives of this study were (i) to compare the changes of fungal abundance and diversity under different upland rice rotations and (ii) determine the factors influencing changes in fungal trophic guilds during upland rice rotation. To achieve the objectives, both phylogenetic and functional group analysis were conducted to assign fungal taxa into three functional guilds: saprotrophs, symbiotrophs and pathotrophs. The hypothesize of this study is that different rice plant rotations will change fungal abundance, diversity, and composition to favor saprotrophs, symbiotrophs or pathotrophs.

## 2. Materials and methods

### 2.1. Site description and management

The long-term upland rice rotation was located at the Irrigation Technology Extension Station of Qingpu District, Shanghai, China (31°12′10″N, 121°08′12″E). The climate of this site was typical subtropical monsoon, with average annual precipitation and temperature of 1300 mm and 17 °C, respectively. The soil is predominantly classified as Fluisols according to the IUSS Working Group WRB (2022), which developed from the soil-forming parent material of river-sea phase sediment. RF, RW and RV rotations were established in 2014, each occupying three replicate plot areas of 5 m × 6 m. An impermeable membrane was buried vertically around each plot to a depth of 1.2-m depth to prevent lateral seepage between plots. From 2014 to 2020, the total fertilizer applied during each season included urea (200 kg $ha^{-1}$), $P_2O_5$ (90 kg $ha^{-1}$), and $K_2O$ (225 kg $ha^{-1}$). The urea was applied as basal fertilizer (50 %), tillering fertilizer (30 %) and heading fertilizer (20 %). The potassium fertilizer was applied in the form of $K_2O$, with about 50 % of the total rate were applied as the basal fertilizer and the remainder was applied as the heading fertilizer. The phosphorous fertilizer was all applied as a basal fertilizer in the form of $P_2O_5$. The wheat and Chinese milk vetch were sown in winter after the rice was harvested at the rate of customary seeding quantities (150 kg/ha for wheat and 75 kg/ha for Chinese milk vetch). All rice and rotation crop plants were removed after each harvest.

### 2.2. Sampling and analysis

Soil samples were collected using a 2.5 cm ∅ drill from the plough layer (0–20 cm) in late November 2020, after the rice was harvested. To minimize within-plot variation, five samples were collected from each plot and mixed uniformly to form one composite sample. Finally, two composite samples from a plot were collected. A 2-mm sieve was used to remove plant residues and gravels, and the residual samples were stored at −80 °C for molecular analyses or air dried at room temperature for chemical analyses.

Soil pH was measured from samples in a water-to-soil ratio of 2.5:1 with a glass-combination electrode [18]. Soil electrical conductivity (EC) was determined at a water-to-ratio of 5:1 using a conductivity meter. Soil organic content (SOC) was analyzed according to the methods of Strickland and Sollins [19]. Soil available N (AN) and available P (AP) were determined according to the methods of Zhang et al. [17] and Olsen et al. [20], respectively. Available K (AK) was assayed using the procedure described by Olsen [20].

### 2.3. PCR amplification and barcode pyrosequencing

The soil's total DNA was extracted using a Soil DNA Isolation Kit (MOBIO Isolation Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. To minimize the DNA extraction bias, three successive soil total DNAs from a soil sample were extracted, and mixed to form a composite DNA sample. The genomic DNA was purified and quality checked as in Wang et al. [13].

Bacterial and fungal abundances were determined by 16S rRNA gene and the fungal internal transcribed spacer (ITS) gene copy numbers, respectively, using a qPCR System (Roche cobas z 480, Germany) with the universal primer pairs 515F/806R to target the bacterial V4 region of 16S rRNA gene [21] and ITS3F/ITS4R for the ITS2 of fungi [22], respectively. The qPCR programs were run as described by Wang et al. [23]. The bacteria-to-fungi ratio was calculated using the 16S rRNA and ITS gene copy numbers as described by Adamczyk et al. [24].

The primer pair ITS3F-ITS4R targeting the fungal ITS2 genes were applied to amplify fungal libraries with TransGen AP221-02 (TransGen Biotech, Beijing, China). The PCR reactions contained 4 μl 5 × FastPfu Buffer, 2 μl dNTPs (2.5 mM), 5 μM of each primer, 0.4 μl FastPfu Polymerase, 0.2 μl Bovinealbumin and 10 ng Template DNA and were adjusted to a final volume of 20 μl with

ddH$_2$O. The PCR reaction was performed with ABI GeneAmp® 9700 (Applied Biosystems, Foster City, CA, USA) set for 95 °C for 3 min with 30 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s, followed by a final extension at 72 °C for 10 min. PCR products were purified and sequenced using the Illumina MiSeq PE 300 platform at the Shanghai Majorbio Bio-pharm Technology Co., Ltd (Shanghai, China).

### 2.4. Sequence analysis

The sequencing data were analyzed using QIIME software (1.9.1) [25]. Sequence reads were filtered using Fastp (0.19.6) according to the following criteria: (1) reads with a quality score <20 over a 3- bp window size, (2) only reads with a more than a 10-bp overlap and less than 20 % mismatches were combined, and (3) the mismatch was less than 0 % and 2 % when sequences were assigned to different sample libraries on the basis of the unique barcodes and primers, respectively. Potential chimeras were removed using the UCHIME software [26]. The remaining high-quality sequences were clustered with UPARSE (7.0.1090) into OTUs with a similarity of more than 0.97. The longest sequence from each OTU was selected to assign taxonomic data from the UNITE database (Ver. 8.0) using RDP Classifier [27]. Sequences present in less than five copies in three or more samples were removed. The fungal alpha-diversity indices, including Shannon, Simpson, Chao1 and ACE, were estimated using QIIME (Version 1.8 http://qiime.org/) software.

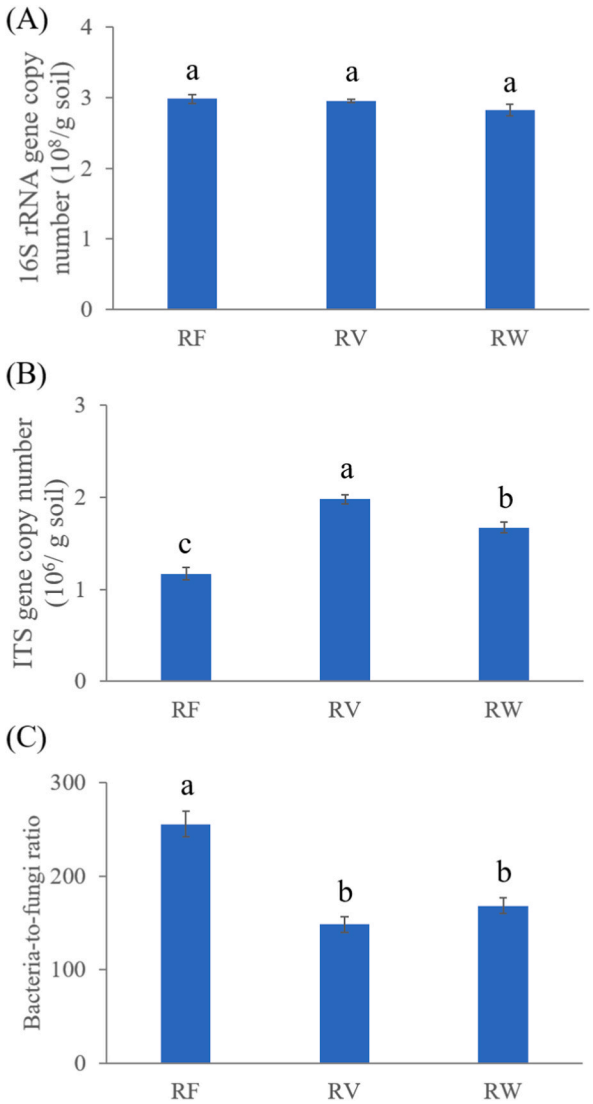


**Fig. 1.** Effects of rice-upland rotations on (a) 16S rRNA gene copy numbers, (b) ITS gene copy numbers and (c) bacteria-to-fungi ratios. Vertical bars represent the standard deviations (n = 6). Different letters indicate significant differences among rice-upland rotation at $P < 0.05$ (Tukey's test).

### 2.5. Statistical analyses

The differences in soil properties, alpha-diversity, community and fungal functional guild abundances among samples were analyzed using one-way analysis of variance. The comparisons of treatment means were determined by Tukey's procedure using SPSS (version 24.0) statistical software (SPSS, Chicago, IL, USA). A principal coordinate analysis (PCoA) was performed to estimate the beta-diversity based on the unweighted UniFrac distances between samples. An analysis of similarities was calculated with 999 permutations in the R "vegan" package based on Bary–Curtis distances. The correlations between environmental variables (pH, Available P, K, N and soil organic content) and the fungal functional guilds community composition were determined using a redundancy analysis (RDA).

Structural equation modeling (SEM) was applied to analyze how crop rotations in upland rice cultivation change soil properties, fungal functional guilds diversity and abundances, and their influence on rice yields. The estimates with $\chi^2/df < 2$, high goodness-of-fit statistics (GFI) indicesand low root-mean squared error of approximation (RMSEA) values were used to determine whether the data fitted the models [28].

## 3. Results

### 3.1. Soil properties

The eight-year upland rice rotation significantly changed the soil pH, EC, SOC, available P, N: P ratio and rice plant height and grain yield, but not the AN or AK (Table S1). RW rotation significantly decreased soil pH by 4.96 % and 4.08 %, respectively, relative to the RF and RV rotations. SOC and available P were increased by RV and RW rotations than RF rotation, and the available P content was higher in RW than RV, while SOC was higher in RV than RW. In contrast, the soil N: P ratio was significantly decreased in RV and RW rotations compared to RF, which decreased by 11.93 % and 26.87 %, respectively. The rice yield was increased by RV and RW rotations by 2.03 % and 1.74 %, respectively, compared to RF rotation.

### 3.2. Microbial abundance and bacteria-to-fungi ratio

The different eight-year upland rice rotations did not show any significant differences in soil bacterial abundance ($P > 0.05$, Tukey's test. Fig. 1A). However, the qPCR analysis of ITS region copy numbers revealed significant ($P < 0.05$, Tukey's test) differences in soil fungal abundance between the rotation types, where the fungal abundances in the RV and RW rotations were increased by 69.24 % and 42.70 %, respectively, relative to the RF rotation (Fig. 1B). The ITS copy number was significantly and positively correlated with available soil P ($P < 0.05$, $r = 0.581$) and SOC ($P < 0.01$, $r = 0.744$). However, the bacteria-to-fungi ratio was significantly decreased in upland rice rotations. Compared to the RF rotation, the RV rotation displayed a decreased bacteria-to-fungi ratio of 41.81 %, while that of the RW rotation decreased by 34.12 % (Fig. 1C). A significant rise in the bacteria-to-fungi ratio was observed with increasing soil N:P ratio ($P < 0.01$, $r = 0.637$), while an opposite trend was detected for available soil P and SOC (Table S2).

### 3.3. Fungal diversity

After sequencing and filtering for quality, a total of 1,012,129 sequences were obtained from the 18 samples, with a mean average length of 336 bp. The Good's coverage values were all above 99 % with a 97 % similarity cutoff. These results indicated that the sequencing depth was sufficient to evaluate the fungal diversity in these soils.

Significant differences in microbial diversity were observed from the Shannon and Simpson indices ($P < 0.05$). The Shannon index was lower and the Simpson index was higher for the RV and RW rotations relative to the RF rotation. The RV rotation showed a higher Shannon index and a lower Simpson index than the RW rotation. Furthermore, there was no significant difference in fungal richness (ACE and Chao 1) between the RF, RV and RW rotations (Table 1). The Shannon and the Shannon even indices were significantly and positive correlated with soil pH ($r = 0.637$ and 0.892) and the N:P ratio ($r = 0.681$ and 0.912), but were significantly negatively correlated with available soil P ($r = -0.907$ and $-0.888$, respectively) (Table S3).

The β-diversity was investigated using PCoA based on unweighted UniFrac distances between samples (Fig. 2). The PCoA based on OTUs (Fig. 2) indicated that PC1 and PC2 contributed 40.25 % and 23.25 % of the variation observed in fungal species composition between the different treatments, respectively. The samples from the RF, RV and RW rotations were clearly separated into three groups in PCoA, indicating that the application of the different upland rice rotations occurred with rotation-specific alterations in the soil fungal composition.

**Table 1**
Fungal alpha-diversity under eight years rice-upland rotation.

| Treatments | OTUs | Shannon | Simpson | Shannoneven | ACE | Chao1 |
|---|---|---|---|---|---|---|
| RF | 638.33 ± 56.18a | 3.96 ± 0.06a | 0.07 ± 0.01c | 0.61 ± 0.01a | 785.49 ± 48.12a | 784.23 ± 41.27a |
| RV | 608.00 ± 45.60a | 3.49 ± 0.07b | 0.10 ± 0.01b | 0.54 ± 0.01b | 754.09 ± 47.20a | 752.25 ± 51.14a |
| RW | 660.50 ± 42.83a | 3.18 ± 0.11c | 0.22 ± 0.02a | 0.49 ± 0.01c | 788.86 ± 37.25a | 791.32 ± 33.08a |

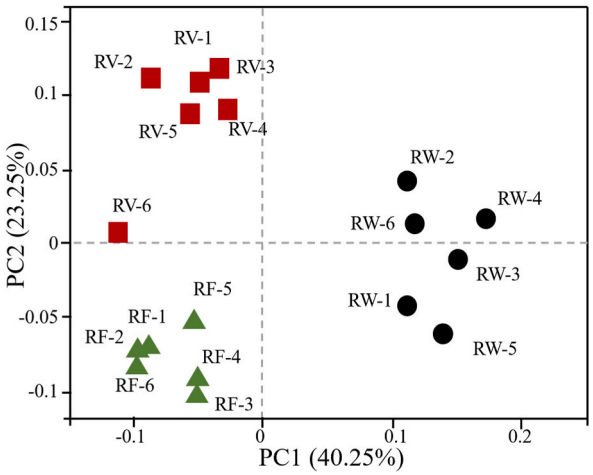


**Fig. 2.** Principal coordinates analysis (PCoA) of the pyrosequencing reads obtained from soils subjected to different upland rice rotation based on the unweighted Fast UniFrac metric.

### 3.4. Fungal composition

The most abundance fungal phylum in the rice upland rotation soils were *Ascomycota* (5.1%–78.0 %), followed by *Basidiomycota* (2.08%–7.01 %), *Mortierellomycota* (0.18%–0.99 %), *Glomeromycota* (0.32%–0.52 %) and *Chytridiomycota* (0.01%–0.04 %). Relative to the RF soil, the relative abundance of *Ascomycota* in the soils from RV and RW rotations was significantly increased by 49.6 % and 35.0 %, respectively. Conversely, the relative abundance of *Basidiomycota* was significantly decreased by 83.05 % and 79.81 % in soils with RV and RW rotations, respectively. Compared to the RF rotation, the RW rotation increased the abundances of *Mortierellomycota* and *Chytridiomycota* spp. by 175.87 % and 56.52 %, respectively, whereas no significant changes were observed in the relative abundance of *Glomeromycota*. In contrast, the soil of the RV rotation showed a decreased abundance in *Glomeromycota* (decreased by 28.65 %), but with no alterations in the abundance of *Mortierellomycota* and *Chytridiomycota* (Fig. S1).

The influence of the different rotation treatments on the fungal composition of rice paddy soils can also be seen at the family level (Fig. 3A-P). Compared to the RF rotation, the RV rotation increased the relative abundance of the *Lasiosphaeriaceae*, *Nectriaceae* and *Helotiaceae* (Fig. 3A–C and H), but decreased the abundances of *Hyaloscyphaceae*, *Hypocreales_fam_Incertae_sedis*, *Chaetomiaceae*, *Exidiaceae*, *Pyronemataceae*, *Protomycetaceae*, *Didymellaceae* and *Trichocomaceae* (Fig. 3B–D, E, F, G, J, L and M).

Compared to the RF rotation, the RW rotation increased the relative abundance of *Pseudeurotiaceae*, *Mortierellaceae*, *Trichocomaceae*, *Didymosphaeriaceae* and *Sporormiaceae* (Fig. 3I–K, M, O and P), while decreases were observed in *Exidiaceae*, *Pyronemataceae*, *Helotiaceae* and *Protomycetaceae* (Fig. 3F, G, H and J).

Compared to the RV rotation, RW rotations showed significant increases in the relative abundances of *Hyaloscyphaceae*, *Hypocreales_fam_Incertae_sedis*, *Chaetomiaceae*, *Pseudeurotiaceae*, *Mortierellaceae*, *Didymellaceae*, *Trichocomaceae*, *Didymosphaeriaceae* and *Sporormiaceae* (Fig. 3B–D, E, I, K, L, O and P), but decreases in *Lasiosphaeriaceae*, *Nectriaceae* and *Helotiaceae* (Fig. 3A–C and H).

### 3.5. Fungal functional guilds

Rice crop rotation had significant influence on the relative abundances of fungal functional guilds (Fig. 4). Compared to the RF rotation, the RV rotation significantly increased the abundances of saprotrophs and pathotrophs by 73.30 % and 32.94 %, respectively (Fig. 4A and C), with a decrease in symbiotroph abundance of 35.96 % (Fig. 4B). In the RW rotation, saprotroph, symbiotroph and pathotroph abundances were all significantly decreased by 33.85 %, 30.97 % and 20.94 %, respectively, compared to RF rotation (Fig. 4A–C). The RV rotation significantly decreased symbiotroph-saprotroph and pathotroph-Saprotroph ratio, compared to RF and RW rotation (Fig. 4D and E). The diversity of functional guilds was also significantly altered by the type of rotation method utilized (Fig. S2). Compared to the RF rotation, the RV rotation significantly increased the diversity of the saprotroph guilds by 34.34 %, while no significant alterations were observed after RW rotations. The opposite trend was observed for the diversity of the symbiotroph guild, where RV rotation had no effect, but those of RW significantly increased symbiotroph diversity by 23.07 %. Concerning the pathotrophic fungi, the RV rotation significantly decreased the diversity in fungal pathotrophs by 23.28 %, while RW rotation caused an increase of 7.07 %.

### 3.6. Factors influencing the composition of fungal functional guilds

The RDA was applied to access the factors that influence the composition of the different fungal functional guilds (Fig. 5). Soil pH, available P, EC and available N were the most important contributors to the variation in the saprotroph guild, explaining 45.62 %, 24.14 %, 6.38 % and 4.89 %, respectively (Fig. 5A). The total contribution from soil factors explained 85.06 % of the variation in

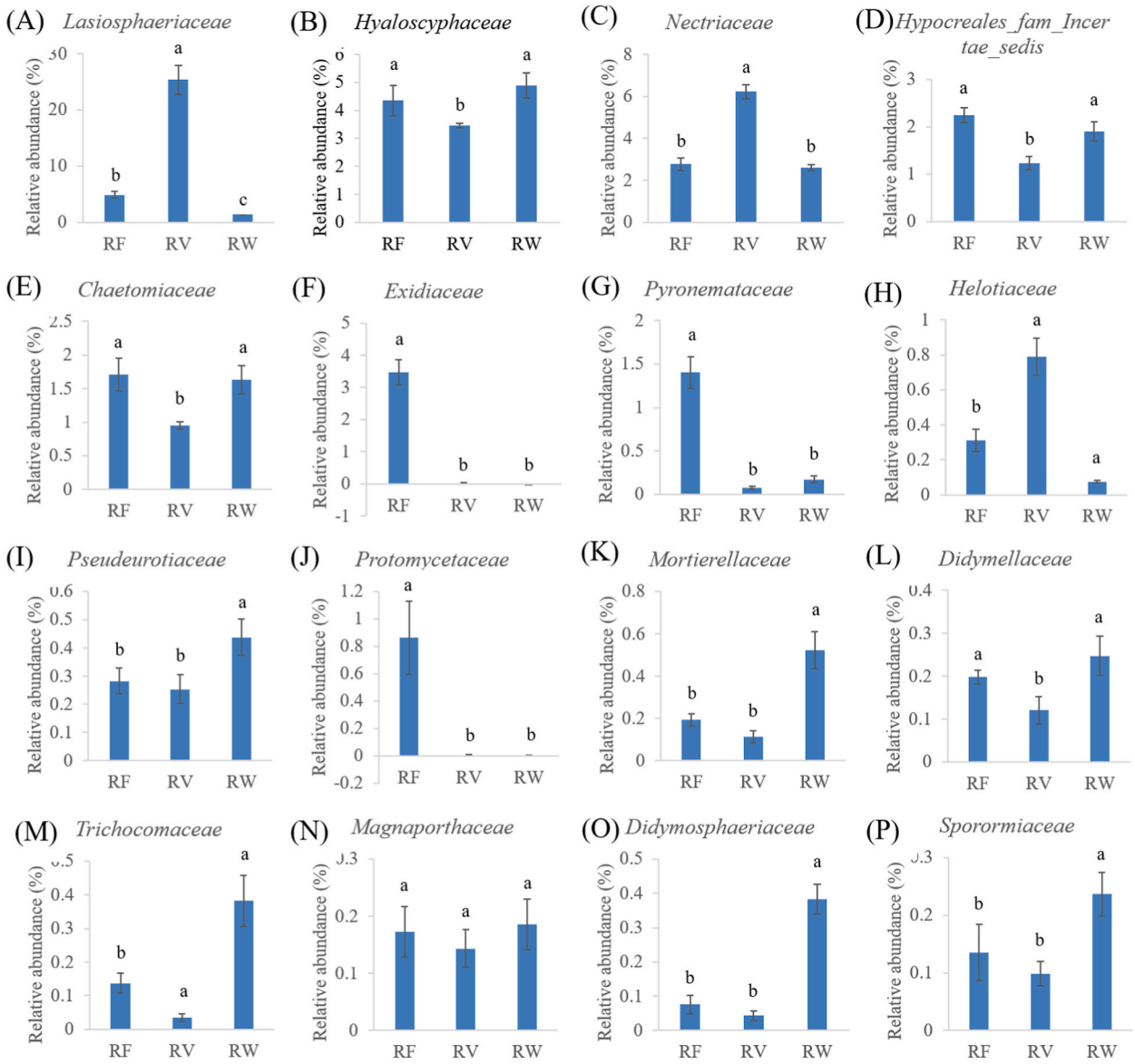


**Fig. 3.** Relative average abundances of the 16 most abundant fungal families in upland rice field soils with different rotation treatments. Vertical bars represent the standard deviations (n = 6). Different letters indicate significant differences among rice-upland rotation at $P < 0.05$ (Tukey's test).

saprotroph guild composition. Similarly, soil pH, available P and EC were the most important factors in explaining variation in the symbiotroph guild (Fig. 5B). Soil pH, available P, EC and available N were the most important factors affecting the pathotroph guild (Fig. 5C).

### *3.7. Relationship between fungal guilds abundance, composition, diversity, and rice yield*

SEM analysis further demonstrated the influence of upland rice rotation treatments on fungal guild composition, abundance, diversity, and on rice yield (Fig. 6; Table S4). The analysis indicated that differences in available P and available N due to upland rice rotation treatments can directly affect rice yield. Soil pH directly influenced saprotroph composition and abundance, while soil available P directly affected symbiotroph and pathotroph compositions, as well as the saprotroph and symbiotroph abundances. Symbiotroph diversity was mainly and directly affected by symbiotroph and saprotroph composition, while pathotroph diversity was mainly affected by symbiotroph and saprotroph abundance. Saprotroph diversity was significantly and directly affected by rice yield in the upland rice rotation ecosystems.

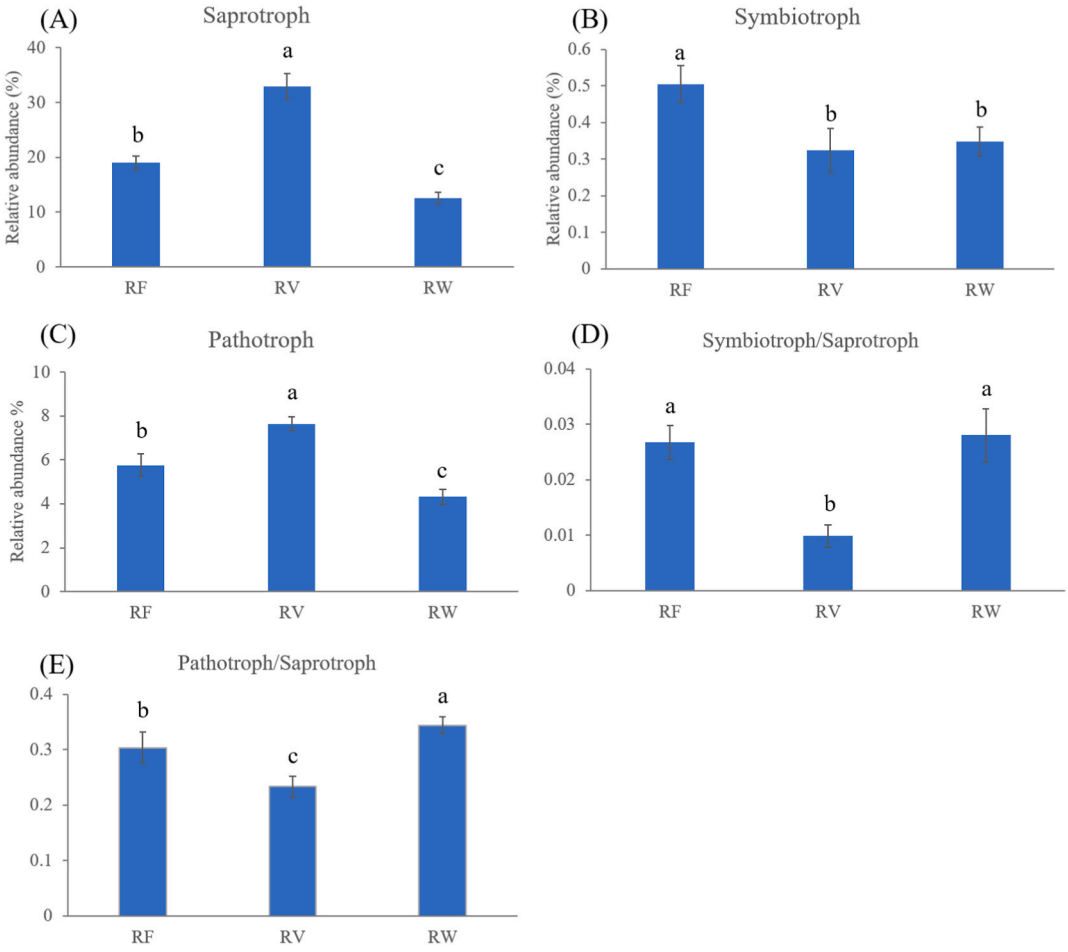


**Fig. 4.** The relative abundance of different fungal guilds under different upland rice rotation treatments. (A) Symbiotroph; (B) Saprotroph; (C) Pathotroph; (D) Symbiotroph-saprotroph ratio; (E) Pathotroph-saprotroph ratio. Vertical bars represent the standard deviations (n = 6). Different letters indicate significant differences among rice-upland rotation at $P < 0.05$ (Tukey's test).

## 4. Discussion

### 4.1. Microbial abundance

In the present study, the results showed that three eight-year upland rice rotation practices did not significantly alter bacterial abundance (Fig. 1A), which is consistent with published results [29]. However, in the present study, upland rice crop rotations with green manure or wheat significantly increased soil fungal abundance relative to the rice-fallow method (Fig. 1B). Previous studies have suggested that fungi can make more efficient use of newly available carbon and plant litter, while bacteria are able to metabolize a wider range of compounds, which may explain their relative stability [30,31]. A recent study showed that fungal C utilization relies mainly on the quantity of recent plant-derived substrates, whereas bacterial access to substrates is additionally controlled by environmental conditions [32]. In the present study, the eight-year upland rice rotation was seen to have a limited effect on soil environment conditions (Table S1). Therefore, this study indicated that the soil fungal composition was more sensitive to upland rice rotation practices than the bacterial composition.

### 4.2. Bacteria-to-fungi ratio

Lower bacteria-to-fungi ratios are frequently considered as a more sustainable agricultural soil system, while fungi dominated systems can enhance plant litter transforming and stabilizing [33]. In most cases, fungi dominate under no-tillage, whereas bacteria dominate under conventional tillage [34]. In this study, the results showed that RV and RW rotations decreased the bacteria-fungi ratio (Fig. 1C), which indicated that upland rice rotation with alternate plant species can directly or indirectly influence the composition of the soil microbial communities. This decrease in ratio was mainly caused by an increase in the fungal abundance because the bacterial abundance remained relatively unaffected between RF, RV and RW conditions (Fig. 1). Previous studies have shown that soil

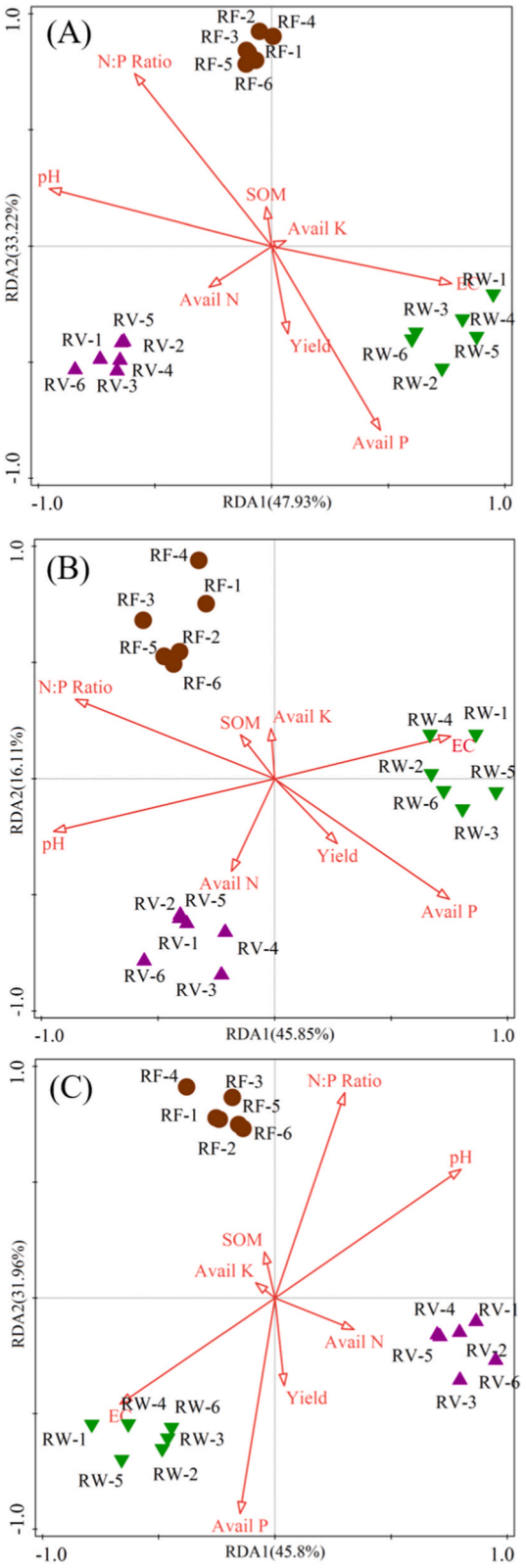


**Fig. 5.** Redundancy analysis (RDA) of different fungal functional guilds under the different upland rice rotation treatments. (A) Saprotroph; (B) Symbiotroph; (C) Pathotroph.

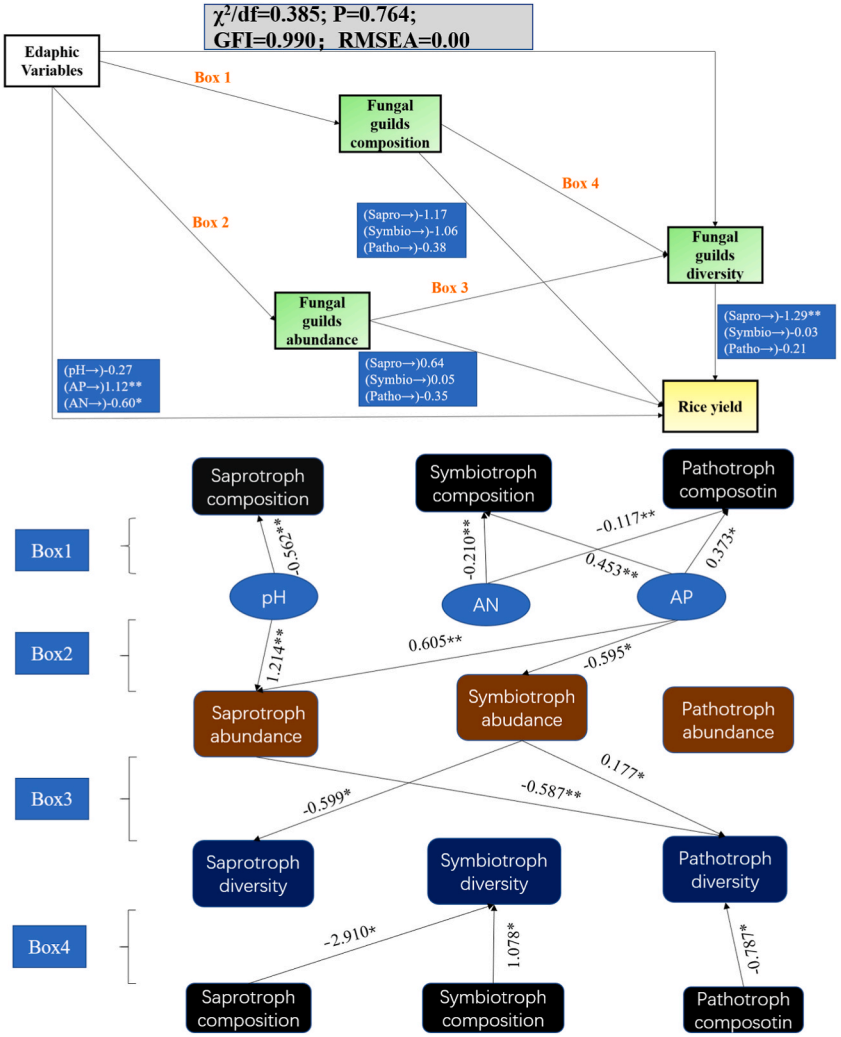


**Fig. 6.** Structural equation modeling (SEM) analysis of the biotic and abiotic factors affecting rice crop production. The direct and indirect effects of different fungal functional guilds abundance, diversity, composition and soil edaphic variables (soil pH, available N [AN], available P [AP]) on crop production. Numbers above/below the arrow lines are indicative of the correlations. The proportion of explained variance ($R^2$) appears alongside wheat production in the model. χ2, Chi-square; df, degrees of freedom; P, probability level; RMSEA, root-mean squared error of approximation are the goodness-of-fit statistics for each model. Significance levels of each predictor are *P < 0.05, **P < 0.01.

bacteria-to-fungi ratio tend to be higher at a lower soil SOC content [15,23]. In agreement, the results showed that soil bacteria-to-fungi ratio was significantly and negatively correlated with SOC ($R = -0.680$; $P < 0.05$). Previous studies have found that plants channel up to 60 % of photosynthetically-derived C to the root system, including tissues, mucilage, or the exudation of organic compounds [35,36]. It can be mainly metabolized by soil fungi rather than bacteria, as fungi have a high ability in assimilating plant derived C, which results in the formation of more complex C compounds with lower degradability [37,38]. In addition, higher abundance of fungi would increase in fungal necromass contribution to SOC pool [39]. These results indicate that fungal dominated soil systems may promote soil C sequestration. In addition, the results showed that soils with RV rotation had a lower bacteria-to-fungi ratio than with RW rotation, and soil organic carbon was higher in RV rotation than RW rotation. Those results suggested that alternating rice and Chinese milk vetch would be a more sustainable rotation system than rice and wheat rotation.

Furthermore, previous studies have shown that high N availability can induce plant allocate more C to favor aboveground tissue production at the expense of root production [15,40]. In the present study, the results showed that RV rotation had a relatively higher concentration of available N with a higher rice yield than from RF and RW rotations.

### 4.3. α-diversity of the soil fungal community

Previous studies have shown that upland rice rotation increases the α-diversity of the bacterial rhizosphere [17]. However, the results showed that RV and RW rotations decreased the soil fungal α-diversity (Table 1). It is important to note that RV and RW

rotations decreased the Shannon diversity and the Shannon evenness indices, but had no significant influence on the fungal Chao1 and ACE richness indices. These results indicated that although the eight-year RV and RW rotations did not cause the elimination of any soil fungal species, these rotations led to drastic changes in the relative abundances of the soil fungal community members. For example, RV rotation dramatically increased the relative abundance of *Ascomycota* by 51.2 %, while RV and RW rotations both strongly decreased *Basidiomycota* abundance (Fig. S1). In addition, the results showed that the fungal α-diversity was higher in RV rotation than in RW rotation. Previous studies have shown that Chinese milk vetch promoted lower soil C/N ratios than wheat straw and that higher C/N ratios had a negative effect on the soil fungal composition [41,42]. This may explain why the eight-year rice-wheat rotation decreased fungal diversity.

The results showed that the rotation of rice with different plants altered the composition and structure of the soil fungal community (Fig. 3). This result was in agreement with other studies that soil fungal communities were strongly determined by plant species [43, 44]. The compositional changes observed may be related to specific alterations in the abiotic or biotic soil environment. In the present study, soil pH and available P were correlated with the diversity and the composition of soil fungal communities. The results showed that *Ascomycota* and *Basidiomycota* were the two most abundant phylum in the rice field soils, which has been observed in agricultural soils in general [15,45]. Previous studies have shown that *Ascomycota* tend to selectively degrade cellulose over lignin, while *Basidiomycota* is more capable of degrading a wider range of complex polymers [46,47]. The promotion of *Ascomycetes* and decrease in *Basidiomycota* spp. by RV and RW rotations observed here could therefore reflect the selection pressure exerted by the availability of more readily degraded cellulose materials in the soil.

In agreement with other studies [15,29], different soil properties were seen to exert different effects on the soil microbial community. For example, soil available P and SOC were correlated with fungal abundance and the bacteria-to-fungi ratio, while soil pH, AP and the N:P ratio shaped fungal diversity and evenness. In addition, soil pH, available P, EC and available N appeared to affect the functional composition of the soil fungal community.

### 4.4. Fungal function guilds

In the present study, the different upland rice rotations differentially affected the diversity (Shannon index) and relative abundance of saprotrophic, symbiotrophic and pathotrophic fungal function guilds in soils (Figs. 4 and 5). Soil fungi belonging to the saprotrophic guild secrete extracellular enzymes to degrade organic matter and play a vital role in nutrient cycling [48]. In this study, the relative abundance of saprotrophtes was significantly increased by the RV rotation (Fig. 4A). A previous study reported that the rotation of rice with Chinese milk vetch manure enhances the decomposition of crop residues [29]. This suggests that rice-Chinese milk vetch rotations could promote soil nutrient cycling through an increase in the abundance of soil fungal saprotrophtes. In contrast, the RW rotation was observed significantly decrease the abundance of fungal saprophytes (Fig. 4A). This may reflect to the need for relatively higher soil N contents for wheat straw decomposition and that lower soil N concentrations can inhibit the growth of saprotrophic taxa [7,45].

Symbiotrophic (mycorrhizal) fungi play important roles in plant development and the modulation of plant–soil interactions [11, 49]. In agreement with recent study [50], both RV and RF rotations significantly decreased the abundance of fungal symbiotrophs (Fig. 4B). For example, the relative abundance of *Archaeosporaceae*, which can form mycorrhizal symbiosis with plants, showed a relatively higher abundance in RF than in RV and RW rotations.

In the present study, the relative abundance of pathotrophic fungi was significantly increased by the RV rotation (Fig. 4C). This may be due to the influence of RV rotation of soil N content since soils from the rice-Chinese milk vetch rotations were seen to have the highest N level (Table S1) and previous studies found that higher soil N levels promote fungal genera with known pathogenic traits [15, 45]. In contrast, rice-wheat rotation decreases the relative abundance of fungal pathotrophs, which may be due to the inhibitory effects of wheat exudates on fungal pathogen growth. Wheat root exudates are rich in benzothiazole and phenolic materials [51], which can increase the abundances of microorganisms potentially antagonistic to the growth of soil fungal pathogens such as *Fusarium oxysporum* f.sp. *niveum* [52].

### 4.5. Factors influencing rice yield

The soil microbial biodiversity plays an important role in soil nutrient cycling, plant production and pathogen control in natural and agricultural terrestrial ecosystems [53–55]. A previous study reported that the diversity in the fungal and arbuscular mycorrhizal fungi soil community was negatively correlated with crop production [55]. In the present study, the diversities of soil fungal saprotrophs, symbiotrophs and pathotrophs was negatively correlated with rice yield (Fig. 6). This is a striking result, as special microbial taxa would enrich in their favorite niche, such as plant rhizosphere would enrich soil R-selected taxa [45], and exert their functions. This assembles progress would decrease soil microbial diversity. In this study, the diversity was lower in RV and RW rotation than RF rotation, also suggested that RV and RW rotation enrich specially fungal taxa.

## 5. Conclusion

An eight years upland rice rotation with wheat or Chinese milk vetch significantly changed soil fungal community composition, and increased fungal abundance with a decrease in fungal diversity and the bacteria-to-fungi ratio. In addition, three fungal functional taxa grouped by their trophic strategies was also changed by different upland rice rotations, with RV rotations increased fungal saprotrophs abundance, while RW rotations decreased saprotrophs and pathotrophs abundance. The results also provide evidence for an effect of fungal diversity on plant production. In particular, the diversity of Saprotroph was significantly and directly affected rice yield in rice-

upland ecosystems. These findings highlight the importance of rice-upland rotation in determining the soil nutrients content, soil microbial composition and crop production, which suggests the possibility to improve rice crop production by applying selected plant rotation systems in intensified agricultural ecosystems.

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## Data availability statement

The datasets generated for this study can be found in the NCBI Sequence Read Archive, PRJNA1035761.

## CRediT authorship contribution statement

**Qingfeng Wang:** Writing – original draft, Software, Methodology, Investigation, Conceptualization. **Deping Zhou:** Software, Methodology, Investigation. **Changbin Chu:** Investigation, Data curation. **Zheng Zhao:** Validation, Software. **Mingchao Ma:** Writing – review & editing, Supervision, Software. **Shuhang Wu:** Writing – review & editing, Validation, Supervision, Funding acquisition.

## 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.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24027.

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