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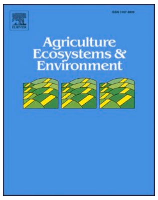

# Straw return improves soil multifunctionality by altering functional microbial diversity and abundance

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## ABSTRACT

Returning straw to the field is widely recognized for its ability to sustain crop productivity and promote the long-term viability of agricultural ecosystems. However, the impact of prolonged straw return on soil functional microbiomes and their relationship with soil multifunctionality (SMF) remain insufficiently understood. This study is based on an 8-year field experiment comprising four treatments: no straw return (N), rice straw return (R), wheat straw return (W), and combined rice and wheat straw return (RW). All straw incorporation methods increased the wheat yield and SMF. Rice straw increased SMF by 69.7 %, while wheat straw enhanced SMF by 52.1 %, with RW exhibiting the most significant long-term positive effect (74.8 %). This enhancement was primarily attributed to elevated available soil nutrient levels and increased enzymatic activities associated with carbon, nitrogen, and phosphorus cycling. Straw return promoted C, N, and P cycling genes following the trend RW > W > R > N. Random forest analysis identified the composition and abundance of functional microbial communities as key determinants of crop productivity. RW markedly diminished the abundance of plant-associated beneficial bacteria and consumers while promoting fungal pathogens in wheat leaves and reducing their prevalence in rhizosphere soil. Potentially beneficial bacteria exhibited a strong predictive capacity for wheat yield, significant associations with soil functionality, and compensatory effects with soil nutrients in their contributions. Structural equation modeling revealed that straw return was significantly positively correlated with enhanced SMF and C-N-P cycling efficiency, ultimately promoting wheat yield. Consequently, these findings suggest that straw return drives microbial community assembly, enhances nutrient cycling, and fosters improved soil fertility and multifunctionality.

## 1. Introduction

With the global population steadily growing, it is projected that food demand will increase by 30 % by 2050, posing a substantial challenge to the maintenance of food security (Cheng et al., 2015). In China, the rice–wheat cropping system has emerged as the predominant agricultural practice, integrating irrigated paddy fields with dryland farming and encompassing approximately 4.67 million hectares (Zhang et al., 2024a). This cultivation method is primarily concentrated in the central and southern regions of the Yangtze River basin (Shi et al., 2021). Although this system achieves high yields of rice and wheat, it also generates a considerable amount of straw as a byproduct, potentially leading to economic and social issues within the agricultural sector. The incorporation of straw into agricultural soils is a widely adopted practice

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among farmers around the world, and it serves as a pivotal strategy for advancing sustainable development. This method, known as straw returning, can effectively augment crop productivity and improve soil quality through altering the composition of soil microbial communities and their associated metabolic pathways (Liu et al., 2023a; Yang et al., 2021). Previous research has demonstrated that soil functional microorganisms and soil ecosystem functions are pivotal in influencing the health of both soil and crops (Lei et al., 2025). However, the long-term effects of straw returning on the functional microbial community and multifaceted agroecosystem functions have not been thoroughly explored in existing research.

Soil microbial communities are essential for straw degradation, a process that depends largely on both the presence of nutrients in the soil and the microbes' ability to utilize these nutrients efficiently (Chen et al., 2023; Pan et al., 2016). The incorporation of straw into the soil has been demonstrated to modify microbial diversity and structure, consequently impacting essential biological functions and influencing nutrient cycling dynamics (Liu et al., 2014). Although a recent meta-analysis suggests that pathogenic fungi introduced through straw incorporation may contribute to the emergence of plant diseases (Yu et al., 2023), current research findings are inconclusive regarding whether the practice of straw return exacerbates the incidence of soil-borne diseases. Moreover, our earlier findings imply that plant-associated microbial communities have a major impact on both crop productivity and quality (Han et al., 2025; Liu et al., 2025; Wu et al., 2022a, 2022b). Soil protists contribute to plant growth and vitality through preying on plant pathogens and modulating the structure and function of the rhizosphere microbiome (Xiong et al., 2020). However, the influence of straw return on the composition of plant-associated microbiota and soil protist communities remains unclear, as do the potential functional consequences for plant health and performance.

Microbial diversity, composition, activity and the roles of these microorganisms are fundamental to biogeochemical cycling, providing crucial contributions to soil health and the long-term sustainability of agricultural output (Fan et al., 2021; Wu et al., 2023). The stability of the soil microbiome and the efficient functioning of its associated processes are critical to maintain the comprehensive service capabilities of soil ecosystems (Han et al., 2023a; Yu et al., 2024). Reduced soil microbial diversity has become a global concern, with significant repercussions for the health of agroecosystems and the essential functions of ecosystems at large (Chen et al., 2020; Yu et al., 2024). Organic fertilization methods have been demonstrated to enhance soil microbiome metabolism and activity via supplying abundant organic matter and essential nutrients, thereby promoting improved soil multifunctionality (SMF) (Han et al., 2022). SMF is assessed in terms of soil functions or biodiversity. Soil microorganisms play pivotal roles in various critical processes, including nutrient cycling, disease suppression, and primary production. Specific microbial groups are particularly vital for maintaining ecosystem multifunctionality (Hu et al., 2024a). However, in agroecosystems, the application of chemical fertilizers has been shown to reduce the capacity for multifunctionality, thereby compromising essential soil functions. SMF is closely related to the functionality of rhizosphere microorganisms (Wu et al., 2023).

Previous studies have demonstrated that incorporating straw into the soil significantly boosts the populations of microbes responsible for processes such as chemoheterotrophy, nitrogen fixation, and key functions in straw decomposition, including chitinolysis, cellulolysis, and xylanolysis (Liu et al., 2024; Zhang et al., 2024b, 2022b). Moreover, straw returning has been shown to suppress the growth of microbes involved in nitrification and nitrate reduction (Zhang et al., 2024b). Straw return can augment the carbon, nitrogen, and phosphorus in topsoil, thereby promoting crop growth and supporting environmental mitigation initiatives (Liu et al., 2023a). Accordingly, in a rotation system involving wheat and maize, it was observed that returning straw to the field promoted bacterial diversity and increased the abundance of functional genes linked to the cycling of carbon, nitrogen, and phosphorus in comparison to the removal of straw (Wu et al., 2023). Functional nitrogen-fixing bacteria influence the nitrogen cycle in ecosystems through stimulating both nitrification and denitrification within the soil (Dai et al., 2020). Microbial communities that participate in carbon cycling facilitate the degradation of organic matter and influence soil carbon storage, making them essential in maintaining the carbon balance in ecosystems (Basile-Doelsch et al., 2020). Microorganisms capable of solubilizing phosphorus facilitate the breakdown of insoluble phosphorus compounds, thereby improving phosphorus bioavailability in the soil (Wang et al., 2023). As such, evaluating the effects of straw return through an integrated framework that accounts for SMF—particularly its critical roles in the biogeochemical cycling of carbon, nitrogen, and phosphorus—may offer a more comprehensive and scientifically sound approach in agricultural systems (Long et al., 2025; Yu et al., 2025). Nevertheless, few studies have thoroughly and concurrently examined how long-term straw incorporation influences overall SMF, specific functional processes, and the associations between key microbial taxa and carbon, nitrogen, and phosphorus cycling. This research aims to bridge this gap by elucidating the correlations among functional microbial communities, soil multifunctionality, and the practice of straw incorporation.

Here, we (1) investigated the effects of different straw returning practices on the plant microbiome, soil physicochemical and biological properties, and soil microbial communities and their associated functional genes in an 8-year field study conducted within a rice–wheat cropping system; and (2) determined the correlation between functional microbial communities and SMF in agroecosystems (in particular, cellulose-degrading bacteria such as *Arthrobacter* and *Pseudomonas*) while identifying the factors that regulate these correlations under the practice of straw return. We hypothesized that (1) returning straw to the soil will support SMF more than straw removal; (2) changes in SMF are associated with carbon, nitrogen, and phosphorus functional microorganisms, with this correlation potentially being influenced by the effects of fertilization on specific soil properties.

## 2. Materials and methods

### 2.1. Field experiments and sampling

The field experiment was carried out at Baihu Farm, located in Lujiang County, Anhui Province, China (31°22′37″N, 117°46′36″E). This area experiences a monsoon-influenced subtropical humid climate, with an average temperature of 16.4°C and annual precipitation of 1150 mm. The initial nutrient concentrations constituting 0–20 cm of soil were as follows: soil organic matter (SOM), 22.3 g $kg^{-1}$; total nitrogen (TN), 1.27 g $kg^{-1}$; available phosphorus (AP), 23.47 mg $kg^{-1}$. The experiment began in 2014, following a summer rice-winter wheat cropping system. Rice was transplanted mechanically in June, while wheat was sown using seed drilling in October. We have set up four treatments, including no straw return (N), rice straw return (R), wheat straw return (W) and combined rice and wheat straw return (RW), all treatments involved a wheat straw incorporation rate of 9000 kg $ha^{-1}$ $y^{-1}$ and a wheat stubble incorporation rate of 6750 kg $ha^{-1}$ $y^{-1}$. Rice and wheat grains were harvested utilizing a harvester equipped with an integrated straw shredder, which processed the straw into segments measuring 6–8 cm in length. The average yield of rice under different treatments ranged from 9175 kg $ha^{-1}$ to 10030 kg $ha^{-1}$. The rice straw was characterized by a total carbon (TC) content of 423 g $kg^{-1}$ and a total nitrogen (TN) content of 12.57 g $kg^{-1}$, whereas the wheat straw exhibited a TC content of 469.36 g $kg^{-1}$ and a TN content of 6.39 g $kg^{-1}$. In May 2021, during the wheat maturation stage, samples of plant and rhizosphere soil were collected for analysis. The rhizosphere soil samples were collected by carefully uprooting wheat plants using a small shovel, then brushing off the soil that was tightly bound to the roots. Samples from each replicate plot were collected from five randomly selected locations and

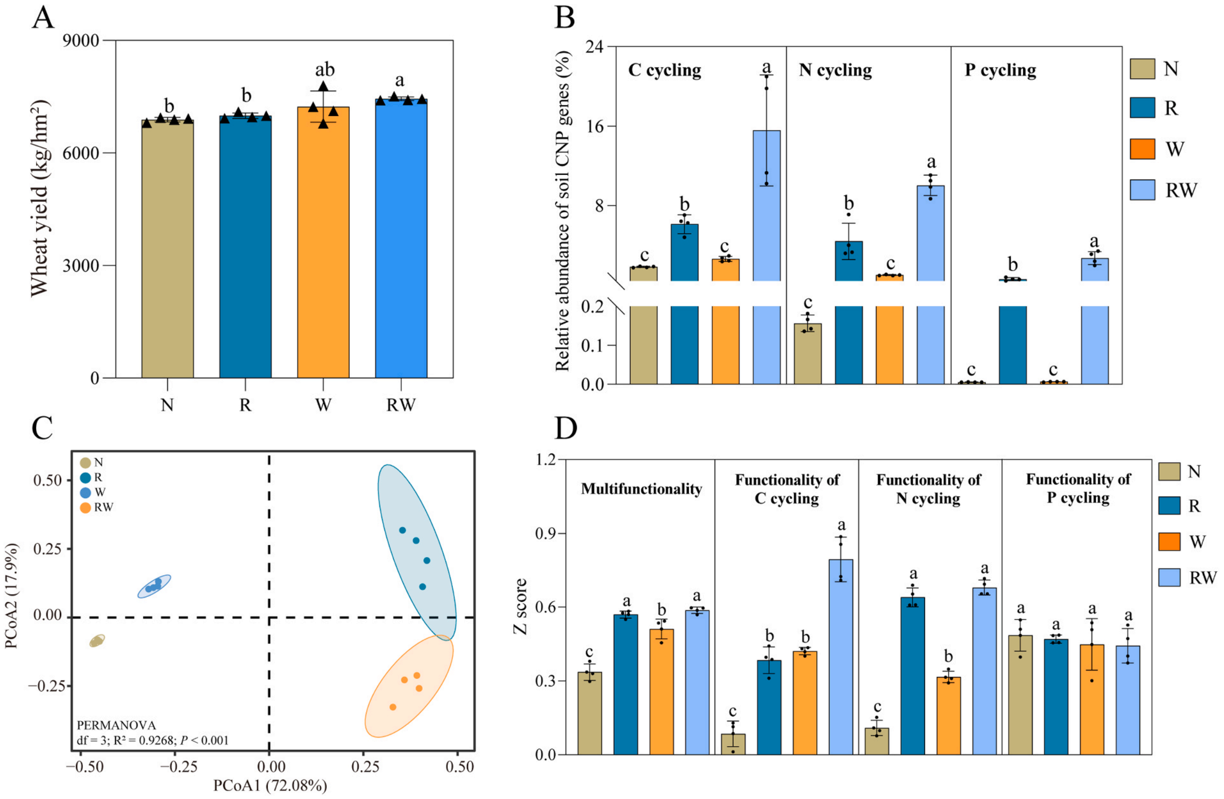

**Fig. 1.** Wheat yields across the different treatments (A). The relative abundance of soil C, N and P cycling genes across all the treatments (B). PCoA analysis of functional genes (C). Soil multifunctionality, along with the cycling functions of carbon, nitrogen, and phosphorus, varied across the different treatments (D). Soil Multifunctionality, Functionality of N cycling, Functionality of C cycling, Functionality of P cycling, in the following text, these terms will be abbreviated as: SMF, FNC, FCC and FPC. Different lowercase letters indicate statistically significant differences among treatments (LSD-test, $p < 0.05$).

subsequently combined to create a composite sample. The collected soil was promptly sieved through a 2-mm nylon mesh to eliminate stones and plant residues, and divided into two portions. Each experimental treatment was performed with four replicates. One portion of the sample was frozen at −80°C for microbial community analysis, whereas the other was kept at ambient temperature, allowed to air-dry, and utilized to evaluate the physicochemical characteristics of the soil. Leaves and roots were sampled from the identical plants used for collecting the corresponding rhizosphere soil samples. To eliminate soil particles that were loosely attached, the plant samples were rinsed three times with sterile water in an oscillator for 20 min. After this washing procedure, the plant samples were blotted dry with sterile filter paper and then flash-frozen in liquid nitrogen. Finally, the samples were stored at −80°C for subsequent analysis.

### 2.2. *Soil physicochemical parameters*

The pH and electrical conductivity (EC) of the soil were measured using a 1:2.5 mass-to-volume soil-water mixture, which was shaken for 30 min at 200 rpm. Measurements were taken using a compound electrode connected to a pH meter (Accumet Excel XL60, Fisher Scientific Inc., Waltham, USA). Soil ammonia nitrogen ($NH_4^+$-N), nitrate nitrogen ($NO_3^-$-N), available phosphorus (AP), DOC (dissolved organic carbon), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN) and microbial biomass phosphorus (MBP) was performed using methods described in earlier research (Khan et al., 2016; Yan et al., 2024a). Solid-acid phosphatase (S-ACP), solid-N-acetyl-β-D-glucosaminidase (S-NAG), solid-β-glucosidase (S-β-GC) activities were assessed using an enzyme spin kit for soil (Beijing Boxbio Science & Technology Co., Ltd., China) and a microplate reader as per the manufacturer's guidelines. All soil property measurements were conducted with four replicates.

### 2.3. *Illumina sequencing and bioinformatic analysis*

DNA was extracted from the roots, leaves, and rhizosphere soil using the Plant DNA Extraction Kit from Omega (USA) and the Soil DNA Extraction Kit from BioFast (China), following the instructions provided by the manufacturers. The extracted DNA's quality and quantity were evaluated using a NanoDrop® ND-1000 spectrophotometer (NanoDrop® Technologies, Wilmington, DE, USA). Libraries of PCR amplicons for the bacterial 16S rRNA gene, fungal ITS region, and eukaryotic 18S rRNA gene were created using universal primer pairs 341 F/806 R (Wang et al., 2019), ITS1F/ITS2R (Adams et al., 2013), and V4_1f/TAReukREV3 (Xiong et al., 2020).

Raw sequences underwent quality control and trimming using the QIIME2 software. Sequences meeting the quality standards were clustered into ASVs at a 97 % sequence similarity threshold. The sequence reads were analyzed through the UPARSE pipeline (Edgar, 2013). The 16S rRNA sequences from bacteria and the ITS sequences from fungi were matched for similarity with the SILVA and UNITE databases, respectively. Simultaneously, the 18S rRNA gene sequences were aligned and compared with the Protist Ribosomal Reference database. The identification of potential beneficial microorganisms was carried out using the global plant-beneficial bacteria database (Li et al., 2023a), while potential fungal pathogens were determined through the FUNGuild database (Nguyen et al., 2016). Based on their feeding behaviors, protist functional groups were grouped at the genus level (Dumack et al., 2020).

### 2.4. *Assessing soil multifunctionality*

A variety of ecosystem functions, including nutrient cycling, soil fertility, and food production, are part of multifunctionality. This

**Table 1**
Soil physicochemical and biological characteristics across all the treatments.

| Treatment | N | R | W | RW | F value | P value |
|---|---|---|---|---|---|---|
| $NH_4^+$-N ($mg \cdot kg^{-1}$) | 0.35 ± 0.03c | 1.23 ± 0.07a | 0.38 ± 0.01c | 0.70 ± 0.02b | 360.32 | < 0.001 |
| $NO_3^-$ -N ($mg \cdot kg^{-1}$) | 109.17 ± 5.66c | 131.17 ± 5.43a | 108.33 ± 2.62c | 120.71 ± 2.76b | 25.20 | < 0.001 |
| AP ($mg \cdot kg^{-1}$) | 49.65 ± 4.18a | 49.65 ± 2.74a | 33.88 ± 2.74b | 26.49 ± 2.47c | 75.91 | < 0.001 |
| DOC ($mg \cdot kg^{-1}$) | 151.33 ± 5.31c | 154.00 ± 4.54bc | 161.33 ± 4.54b | 267.00 ± 7.71a | 413.06 | < 0.001 |
| pH | 5.07 ± 0.08b | 5.05 ± 0.01b | 5.18 ± 0.01a | 5.15 ± 0.03a | 8.36 | 0.0057 |
| EC ($\mu s \cdot cm^{-1}$) | 137.67 ± 6.12a | 135.67 ± 4.49a | 126.33 ± 2.05b | 127.67 ± 5.31b | 40.56 | < 0.001 |
| MBC ($mg \cdot kg^{-1}$) | 36.00 ± 3.55c | 57.33 ± 9.97b | 72.33 ± 5.31a | 57.67 ± 5.79b | 13.00 | < 0.001 |
| MBN ($mg \cdot kg^{-1}$) | 0.57 ± 0.11b | 0.50 ± 0.04b | 1.26 ± 0.19a | 1.18 ± 0.16a | 31.70 | < 0.001 |
| MBP ($mg \cdot kg^{-1}$) | 116.80 ± 13.18b | 72.50 ± 4.50c | 205.18 ± 9.67a | 47.43 ± 2.56d | 98.60 | < 0.001 |
| S-β-GC ($U \cdot g^{-1}$) | 270.41 ± 26.69c | 362.45 ± 5.68b | 355.45 ± 17.07b | 411.52 ± 4.71a | 74.35 | < 0.001 |
| S-NAG ($U \cdot g^{-1}$) | 1.44 ± 0.37d | 3.82 ± 0.20a | 2.41 ± 0.08c | 3.53 ± 0.26b | 193.95 | < 0.001 |
| S-ACP ($U \cdot g^{-1}$) | 3909.67 ± 46.46a | 3930.31 ± 37.53a | 3775.23 ± 39.33a | 4109.64 ± 12.50a | 1.51 | 0.2773 |

N represents no straw return, R represents rice straw return, W represents wheat straw return, RW represents rice and wheat straw return, AP represents available phosphorus, DOC represents dissolved organic carbon, MBC represents microbial biomass carbon, MBN represents microbial biomass nitrogen, MBP represents microbial biomass phosphorus. The values were presented as mean ± Standard Deviation (SD). Significant differences are represented by the various letters in each line (LSD's test, $p < 0.05$, n = 4).

research evaluated the ecosystem functions linked to the cycles of carbon, nitrogen, and phosphorus, along with the overall multifunctionality of soils. We employed quantitative microbial element cycling (QMEC) to perform a high-throughput, quantitative evaluation of functional genes associated with the biogeochemical cycles of Carbon (C), Nitrogen (N), and Phosphorus (P), each treatment had four replicates. Details of measurements and the primer information are provided in previous study (Chen et al., 2020; Zheng et al., 2018). A total of ten soil physicochemical indicators, including $NO_3^-$-N, $NH_4^+$-N, AP, DOC, MBC, MBN, MBP, S-ACP, S-NAG, and S-β-GC activity, along with QEMC data, were utilized in this study. Data standardization was performed using the 0–1 normalization method. The multifunctionality was then calculated using the averaging approach (Hu et al., 2021). Specifically, the functional of carbon cycling (FCC) was determined as the average of the standardized DOC, MBC, S-β-GC activity, and the abundance of carbon cycle-related functional genes. Similarly, the functional of nitrogen cycling (FNC) was calculated as the average of the standardized $NO_3^-$-N, $NH_4^+$-N, MBN, S-NAG activity, and the abundance of nitrogen cycle-related functional genes. The functional of phosphorus cycling (FPC) was derived from the average of the standardized AP, MBP, S-ACP activity, and the abundance of phosphorus cycle-related functional genes. Subsequently, the average of FCC, FNC and FPC was obtained as the SMF.

### 2.5. Statistical analysis

Statistical analysis was performed with the R software environment, version 4.4.1, unless otherwise indicated. In the present study, soil multifunctionality was calculated using the data for soil physicochemical properties (including DOC, $NO_3^-$-N, $NH_4^+$-N, AP), soil enzyme activities (S-ACP, S-NAG and S-β-GC), and microbial biomass (MBC, MBN, MBP). The functionality of C, N, and P cycling was assessed utilizing data pertaining to soil nutrient concentrations, enzyme activities, and microbial biomass. The Shannon index was used to evaluate the α diversity of soil microbes, which is accessible through the "vegan" package in PCoA following Bray–Curtis distance matrix was used to assess differences and correlation in soil microbial structure. Spearman correlation analysis was conducted using the "Hmisc" package and the results were visualized using heatmap. Co-occurrence networks were constructed based on a strong Spearman correlation coefficient ($r = 0.8$, $p = 0.05$) using the "igraph" package, and the networks were visualized with Cytoscape (version 3.7.1) (Han et al., 2023b). Random forest regression was performed using the randomForest package in R. The response relationships between functional microbial abundance and yield were fitted through random forest (i.e., constructing random forest regression models) by using the rfPermute function (Jiao et al., 2018). To evaluate the stability of microbial communities in soil, the Average Variation Degree (AVD) was used as a measure of deviation. In this study, we employed a structural equation model (SEM) utilizing Amos 21.0.0 software (IBM SPSS) to examine the direct and indirect effects of straw incorporation on soil multifunctionality, soil carbon-nitrogen-phosphorus cycling functions, functional microbial abundances, and wheat yield. The least significant difference (LSD) multiple range test was used to determine the significance difference between treatments.

## 3. Results

### 3.1. Crop yields and soil characteristics

Wheat yield increased notably with straw returning, especially with both rice and wheat straw (Fig. 1A). The rice straw return (R) and combined rice and wheat straw (RW) treatments significantly showed higher $NO_3^-$-N and $NH_4^+$-N contents compared to the control ($p < 0.05$). The RW and wheat straw return (W) treatments had significantly higher dissolved organic carbon (DOC), pH, and microbial biomass nitrogen (MBN) than the control, while the AP and EC were reduced. Straw return methods significantly increased the levels of MBC, S-β-GC, and S-NAG compared to the control with the RW treatment having the highest DOC and S-β-GC levels (Table 1).

The soil C-N-P functional gene abundance varied significantly across the different methods of returning straw to the soil (Fig. 1C, Table S1). The abundance of each C-N-P cycling gene exhibited a distinct pattern of variation depending on the type of straw incorporated into the soil (Fig. 1B). The RW treatment had the highest abundance of C-N-P genes compared to the other treatments. SMF and the cycling of carbon and nitrogen were significantly enhanced under straw returning compared to the control (Fig. 1D). The abundance of each functional gene exhibited a unique response to straw return (Fig. 2A). The R and RW treatments led to significant improvements in both SMF and nitrogen cycling functionality compared with the other treatments. Furthermore, the RW treatment exhibited the greatest enhancement of C cycling functionality. Heat-map analysis revealed that the FNC, FPC, and some soil physicochemical and biological characteristics, especially yield, AP, DOC, pH, S-NAG, and S-ACP, exhibited significantly positive correlations with the abundances of most C-N-P cycling genes (Fig. 2B). The co-occurrence networks of C-N-P cycling gene abundances and soil physicochemical and biological characteristics showed multiple associations, dominated by positive edges (Fig. 2C).

### 3.2. Changes in microbial communities under straw returning

Straw returning had less effect on the Chao 1 and Shannon diversity indices of leaf, root, and soil bacterial communities (Figure S1A).

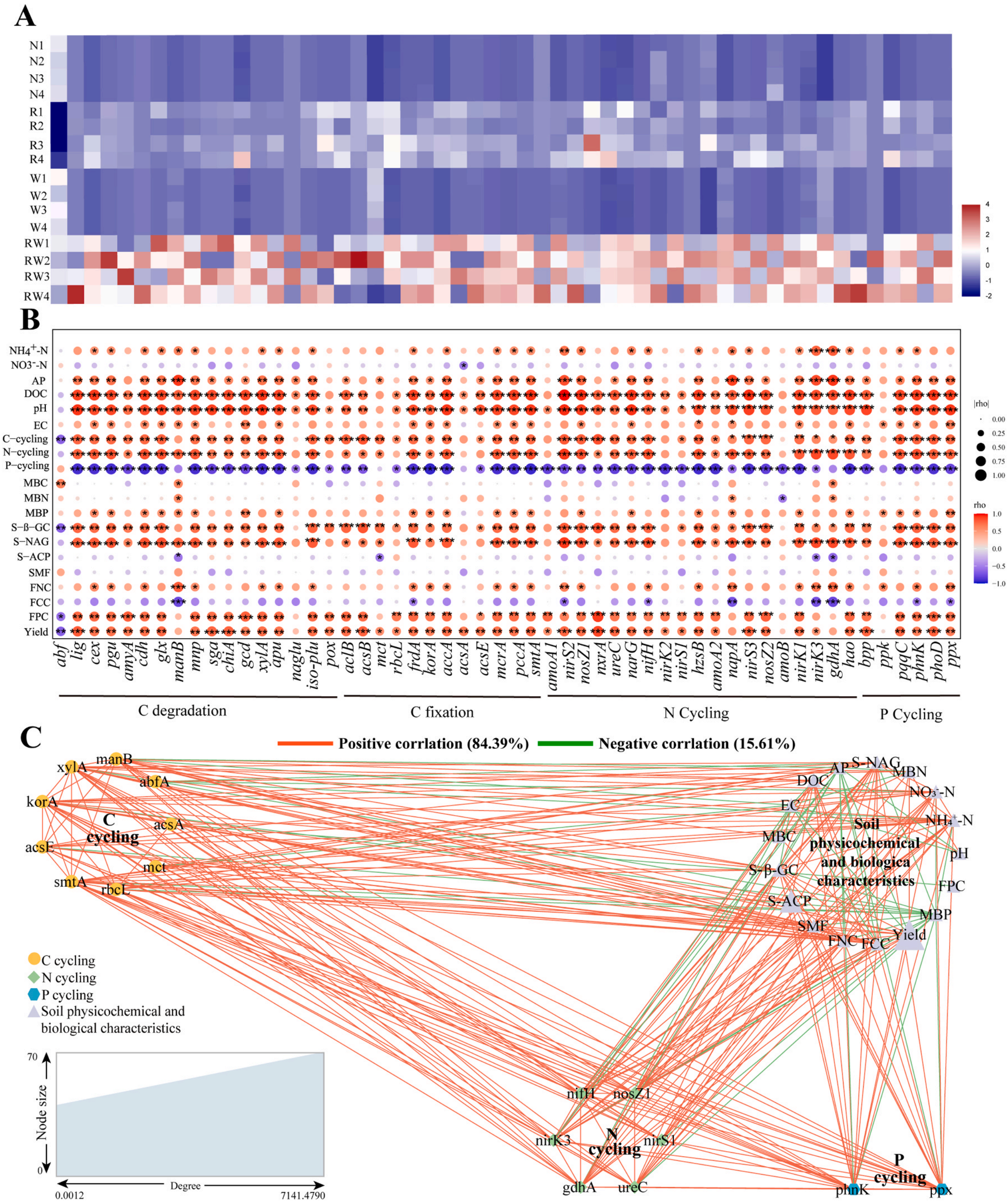

**Fig. 2.** The correlations between functional genes abundances and the soil physicochemical and biological characteristics(A). Alterations in the abundance of functional genes observed across the various treatments (B). Network analysis examining the simultaneous abundance of functional genes and soil physicochemical and biological characteristics utilizing Spearman's correlation analysis (C). Significant differences are represented by the different letters in each column ($p < 0.05$). $***p < 0.005$, $**p < 0.01$.

However, the soil fungal community in the RW treatment had the highest α diversity (Figure S1B). The community structure of soil bacteria and fungi was influenced by straw returning (Figure S1C and D). Furthermore, the W and RW treatments decreased the α diversity of the soil protozoan community (Figure S2). The RW treatment notably enhanced the average variation degree (AVD) within the leaf bacterial community (Fig. 3A). The reintroduction of straw had a significant positive effect on the average variation degree (AVD) of the soil fungal community. Conversely, straw incorporation adversely affected the average niche breadth of bacterial communities in both leaves and soil (Fig. 3B). The RW treatment significantly increased the average niche breadth of soil fungal communities. All treatments exhibited βNTI values below 2, which indicated that stochastic processes predominantly influenced the assembly of both bacterial and fungal communities (Fig. 3C).

The RW treatment significantly enhanced the α diversity of potentially beneficial bacteria in the leaves (Fig. 4A), increased the α diversity of potential fungal pathogens in the soil (Fig. 4B), and had less effect on the α diversity of the consumer protist community (Fig. 4C). Furthermore, straw return had a relatively minor impact on the community composition of beneficial bacteria, fungal pathogens, and consumer protists (Figures S3, S4, and S5; Tables S2, S3, and S4). Random forest

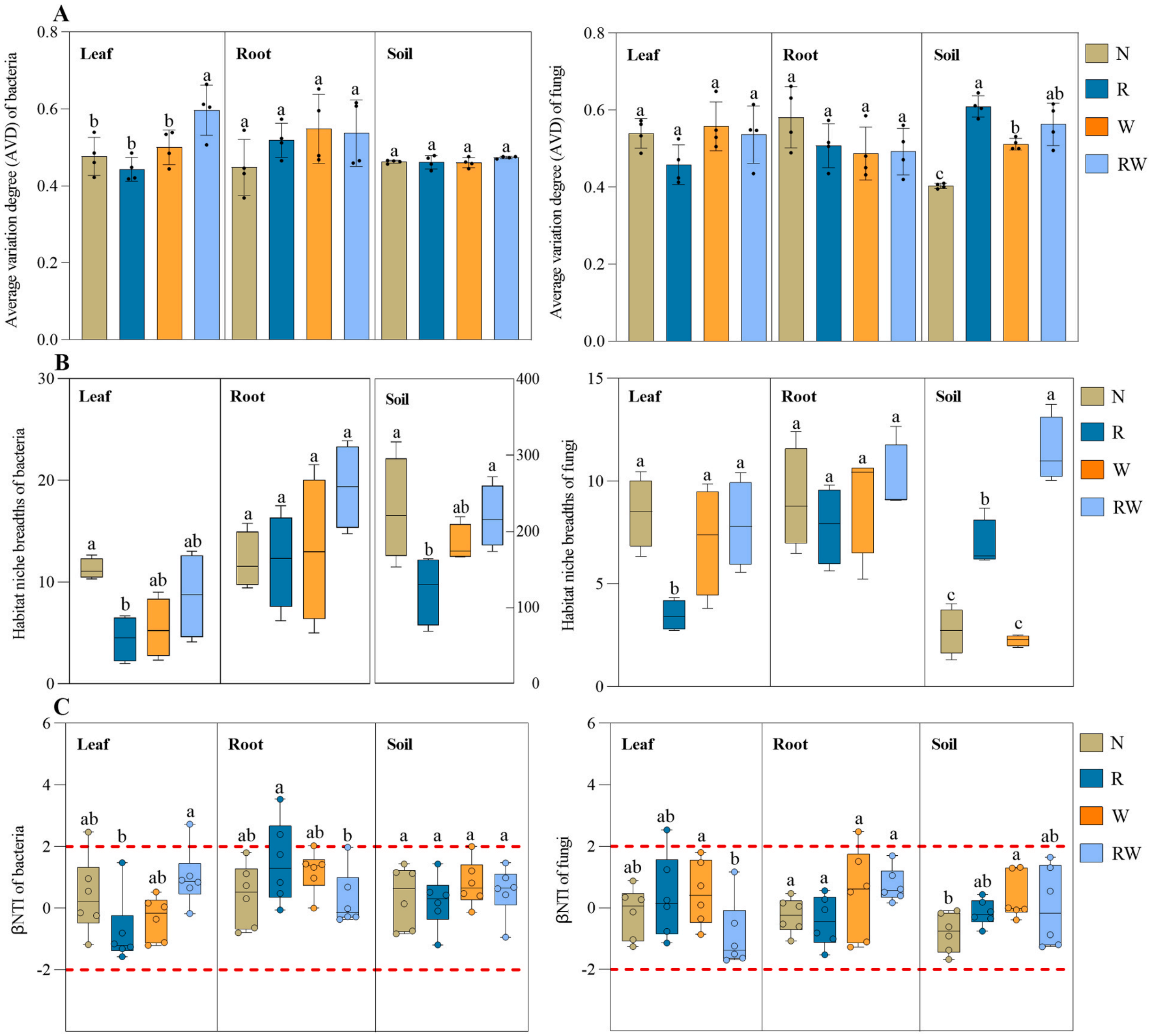

Fig. 3. The average variation degree (AVD) of bacterial and fungal communities in the leaf, root, and soil was assessed separately (A). The niche breadth of bacterial and fungal communities in the leaf, root, and soil was assessed individually (B). The βNTI values for the assembly processes of bacterial and fungal communities in the leaf, root, and soil were determined separately (C). Different lowercase letters indicate statistically significant differences among treatments (LSD-test, $p < 0.05$).

analysis revealed that the diversity and composition of the soil fungal community were the most significant predictors of wheat yield (Fig. 4D), while the relative abundances of beneficial bacteria, fungal pathogens, and consumer protists were also highly predictive of wheat yield (Fig. 4E).The diversity of fungal pathogens and consumer protists was the most significant factor in predicting wheat yields (Fig. 4E). The RW treatment substantially reduced the relative abundances of beneficial bacteria in the leaf, root, and soil communities, especially *Pseudomonas* when compared to the no straw return (N) treatment (Fig. 5A). In addition, the RW treatment substantially decreased the relative abundances of *Arthrobacter*, *Sphingomonas*, *Bradyrhizobium*, *Blastococcus*, *Nitrosospira*, and *Bacillus* compared to the N treatment. Moreover, the W and RW treatments significantly increased the abundance of potential fungal pathogens in the leaf tissue, particularly *Mycosphaerella* and *Alternaria*, compared to the other treatments (Fig. 5B). Conversely, the R and RW treatments led to a significant reduction in the abundance of fungal pathogens in the soil relative to the other treatments.

Our findings indicated that straw returning significantly altered the functional composition of soil protists (Fig. 4C). A significant negative impact on the relative abundance of consumer protists was observed under the RW treatment, with particularly notable declines in the abundances of *Trinema*, *Viridiraptor*, *Aphanomyces*, *Rhogostoma*, *Cercomonas*, and *Colpoda* compared to the N treatment. Both phototrophic and parasitic protists were less abundant under the RW treatment compared to the N treatment (Fig. 5C).

### 3.3. Microbial community changes mediate SMF and yield enhancement

Co-occurrence network analysis revealed that the associations between consumer protists and beneficial bacteria were predominantly positive. This included taxa such as Actinobacteria, Proteobacteria, and Cercomonadidae (Fig. 6A, Table S5). The interactions between beneficial bacteria, consumer protists, and fungal pathogens were mainly defined by negative correlations. Moreover, Mantel tests were employed to explore the associations between α and β diversity within soil functional microbial communities (Fig. 6B–E) and different soil properties. The results of Mantel's test revealed that the soil DOC, pH, and S-NAG represented the drivers shaping the diversity patterns of potentially beneficial communities. In contrast, the diversity of potential fungal pathogens was primarily determined by functional indicators related to carbon, nitrogen, and phosphorus cycling (Mantel's $r > 0.5$, $p < 0.001$). Furthermore, S-ACP and P-cycling genes were found to be the key

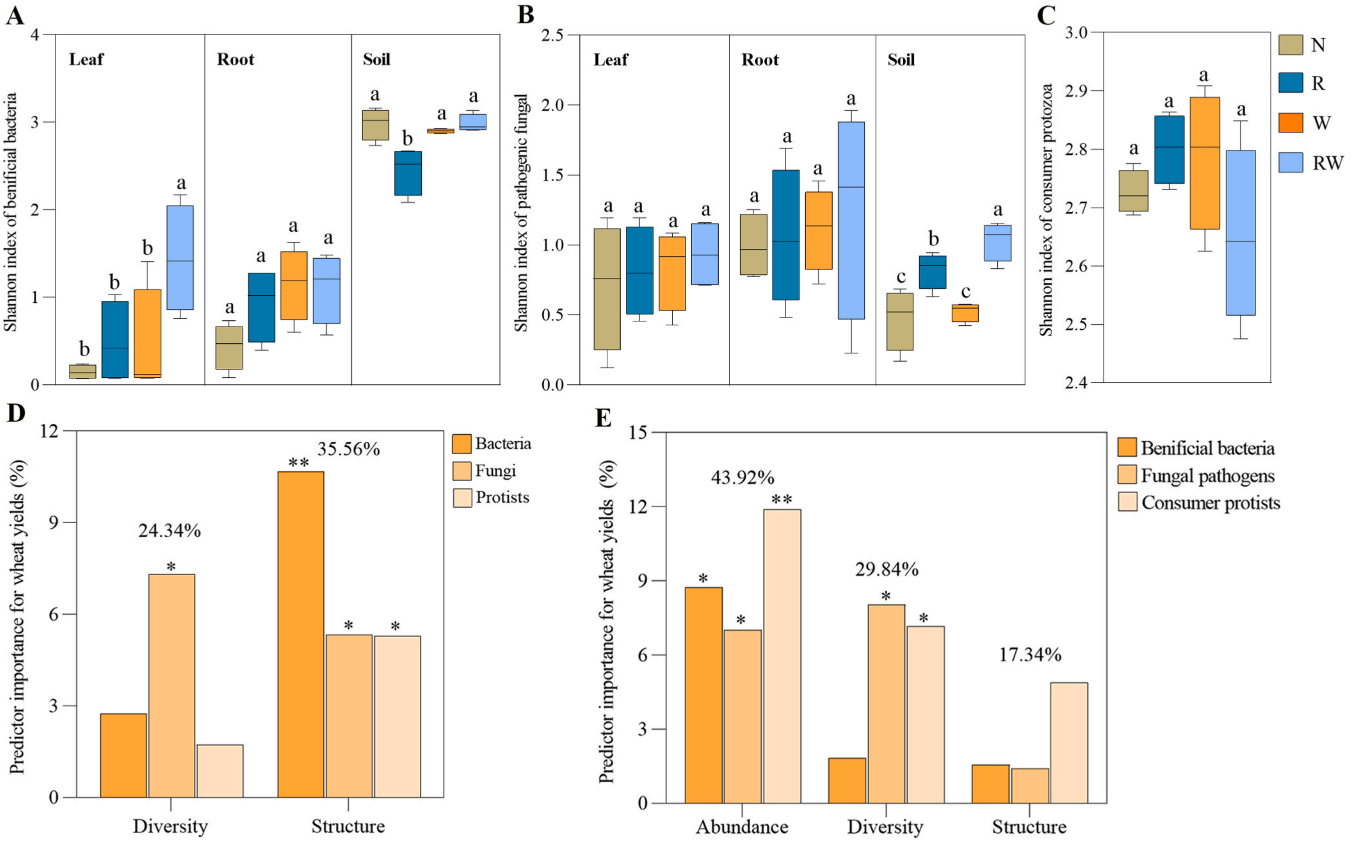

**Fig. 4.** Shannon diversity of beneficial bacteria (A), fungal pathogens (B), consumer protists (C) community, respectively. Importance of bacterial, fungal, and protistan community diversity and structure in predicting wheat yields using Random Forest (% increase in MSE) (D). Importance of beneficial bacteria, fungal pathogens, consumer protists community abundance, diversity and structure in predicting wheat yields (E). Different lowercase letters indicate statistically significant differences among treatments (LSD-test, $p < 0.05$).

factors that significantly influenced the consumer protists communities (Mantel's r > 0.5, $p < 0.05$). The relative abundance of beneficial bacteria exhibited a significant positive correlation with MBP while demonstrating a negative correlation with DOC and FCC (Figure S6). Additionally, the relative abundance of fungal pathogens was negatively associated with C-cycling genes and S-β-GC. Furthermore, the relative abundance of consumer protists exhibited a significant negative relationship with $NH_4^+$-N, $NO_3^-$ -N, S-β-GC, S-NAG, SMF, and FNC.

Random forest analysis revealed that Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes, Firmicutes, Ascomycota, and Basidiomycota were key predictors of SMF (Fig. 7A). Many of these beneficial bacteria and potentially pathogenic fungi are closely linked to the cycling of carbon and nitrogen in soil ecosystems (Fig. 7B). Positive links were identified between C-cycling, N-cycling, S-β-GC, S-NAG, FNC, and FCC with microbial taxa such as *Lysinimonsa*, *Lechevalieria*, *Monilinia*, *Ustilaginoidea*, *Coniochaeta*, and *Ganoderma*. Additionally, soil protists, including Cercozoa, Oomycetes, Heterophryidae, Ciliophora, Acanthocystidae, and Leptomyxida were also identified as significant contributors to SMF, with most showing negative correlations with specific carbon and nitrogen processes. Structural equation modeling further confirmed the vital importance of straw incorporation for sustaining soil functions and regulating carbon and nitrogen dynamics (Fig. 7C). The structural equation model revealed both direct and indirect associations between SMF and wheat yield, as well as between microbial community structure and soil variables. SMF correlated to the functionality of C, N, and P showed the largest direct effect on the yield. The presence of potentially beneficial bacteria, fungal pathogens, and consumer protists had a direct impact on C, N, and P cycling (Fig. 7 C and S7). The overall positive influence on SMF was most strongly driven by the high levels of beneficial bacterial and consumer protist populations.

## 4. Discussion

### 4.1. Straw returning improves ecosystem functions

In this study, an 8-year field experiment demonstrated that the practice of straw return led to significant improvements in various individual ecosystem functions. Our findings further indicated that straw incorporation significantly elevated the levels of available nitrogen and carbon within the soil. Additionally, wheat yield was notably improved, particularly under the RW treatment, in which the wheat yield increased by 8.07 % compared to the N treatment. Straw return has been shown to enhance soil nutrient availability through influencing microbial activity and metabolism, which in turn promotes plant growth, alters root structure, and boosts photosynthesis (Fan et al., 2022; Wei et al., 2025). This process ultimately improves nutrient uptake, increases biomass, and enhances crop yield (Choudhury et al., 2014). Our findings revealed that long-term straw return significantly changed the composition of carbon, nitrogen, and phosphorus cycling genes. Notably, rice straw and rice–wheat straw return increased the abundance of these genes (Fig. 1A–B), which are strongly positively correlated with yield (Fig. 2B). Interestingly, combined rice and wheat straw return exerted more pronounced effects on rhizosphere soil nutrients and functional genes compared to the application of a single type of straw.

Our results show that returning straw to the field improves SMF and yields via regulating C-N-P cycling processes. Among nutrient cycling genes, the abundance of functional genes including *smtA*, *manB*, *xyLA*, *ascA*, *acsE* and *rbcL* were significantly increased after straw return, which are associated with the degradation of hemicellulose, starch and cellulose (Li et al., 2023b). Meanwhile, straw return enhanced the abundance of genes involved in N fixation (*nifK*), denitrification (*nirK*,

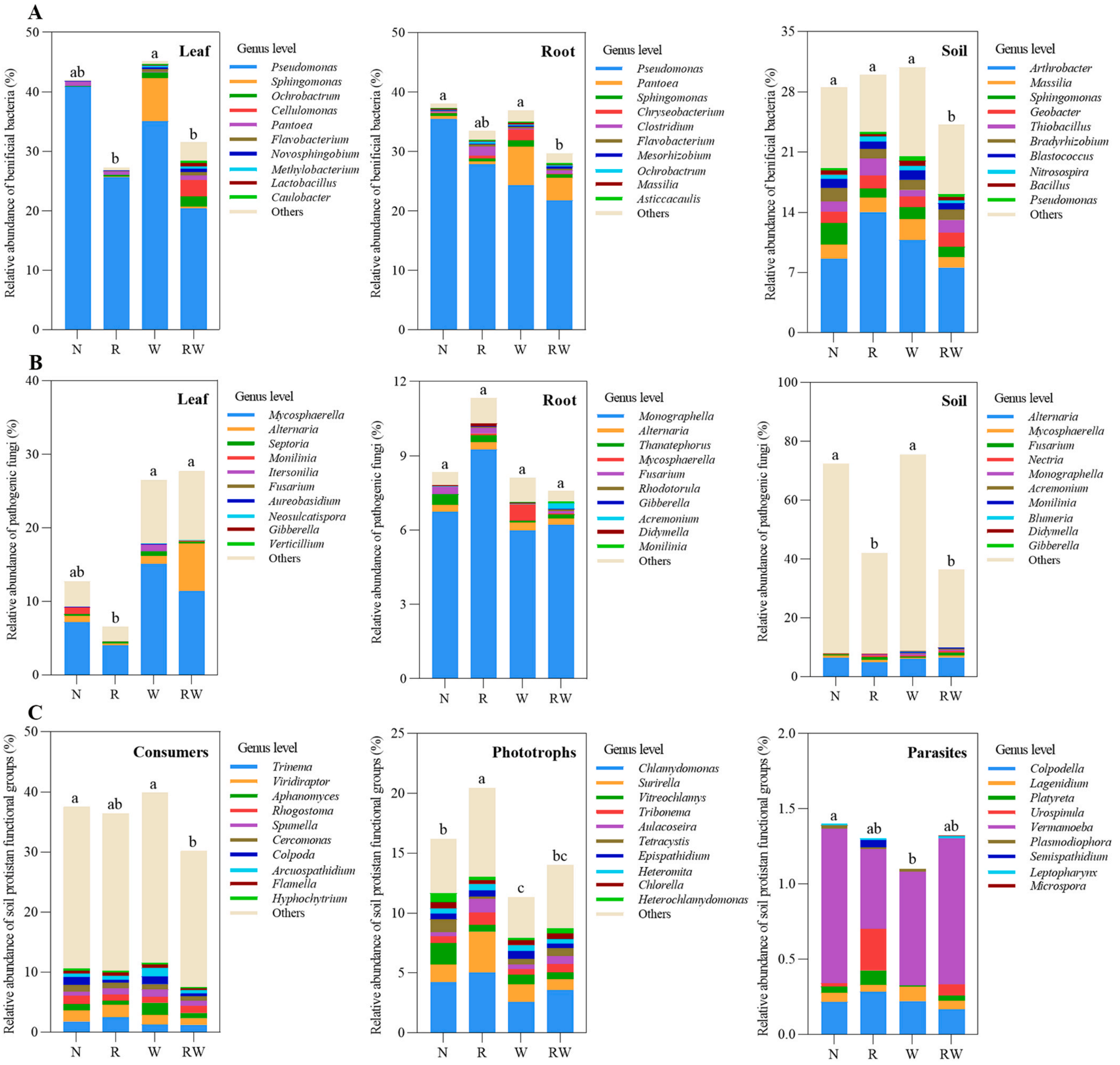

**Fig. 5.** The relative levels of potentially beneficial bacteria (A) and of fungal pathogens at the genus level (B) in the leaf, root and soil. Distribution of the relative abundance of functional groups of soil protists (C). Different lowercase letters indicate statistically significant differences among treatments (LSD-test, $p < 0.05$).

*nosZ*, *narG*, and *napA*), and nitrification (*amoB* and *hao*), indicating a substantial boost in the soil C-cycling and N-cycling potential. Previous studies showed that the straw return enhances microbial growth by increasing the availability of key nutrients such as organic carbon and nitrogen, while strengthening soil carbon and nitrogen sequestration capacity (Dong et al., 2019). Our field-based data demonstrate that the practice of straw return significantly enhances DOC sequestration in soils (Figs. 1 and 2), primarily as a result of the integration of straw-derived carbon into microbial cells (Sun et al., 2016; Zhang et al., 2022a). Straw return has been shown to increase soil carbon storage and its bioavailability, consequently promoting microbial activity and the synthesis of enzymes involved in carbon breakdown (Wu et al., 2024), which aligns with the increase in MBC observed in our study.

Our results also showed that long-term straw return led to a considerable elevation in the soil $NH_4^+$-N and $NO_3^-$-N contents, stimulating the activity of key N-cycling genes (*nifH*, *nosZ1*, and *ureC*) (Table 1, Fig. 1). As straw decomposes, it continuously releases nutrients into the soil, sustaining long-term increases in $NH_4^+$-N and $NO_3^-$-N levels (Xie et al., 2017). In the present study, among genes encoding functions involved in phosphorus cycling, the abundance of genes associated with organic phosphorus mineralization (*phoD* and *phnK*) and inorganic phosphorus dissolution (*ppx*) increased, while the phosphorus content of rice and wheat straw was relatively low. However, microorganisms require phosphorus to decompose straw. This could potentially reduce the available phosphorus levels in the soil (Yadvinder et al., 2005), which may explain the decreased AP content observed in our study (Table 1). Furthermore, in the present study, the available phosphorus content in the soil reached levels sufficient for normal wheat growth, and it was not a limiting factor for wheat development (Hou et al., 2023; Yan et al., 2024b). Overall, straw returning can enhance soil nutrient cycling function.

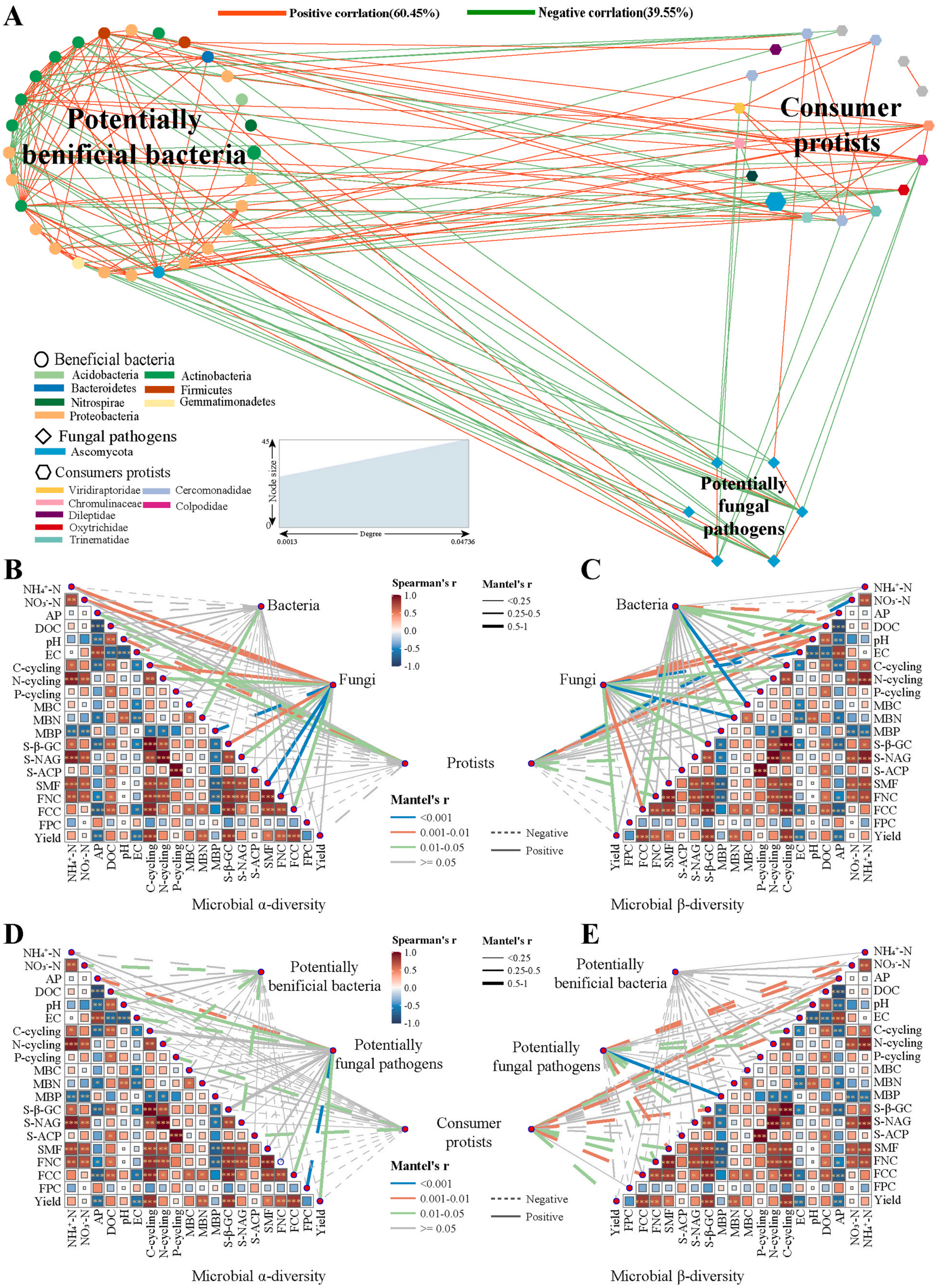

**Fig. 6.** Co-occurrence network was constructed to examine the interactions among genera of beneficial bacteria, pathogenic fungi, and consumer protists, based on Spearman correlation analysis (A). Mantel tests were employed to reveal the relationship between microbial alpha diversity (B) and β diversity with soil variables (C), respectively. Mantel tests reveal the linkage between functional microbial α diversity (D) and β diversity (E) with soil variables, respectively. Significant differences are represented by the different letters in each column ($p < 0.05$). ***$p < 0.005$, **$p < 0.01$.

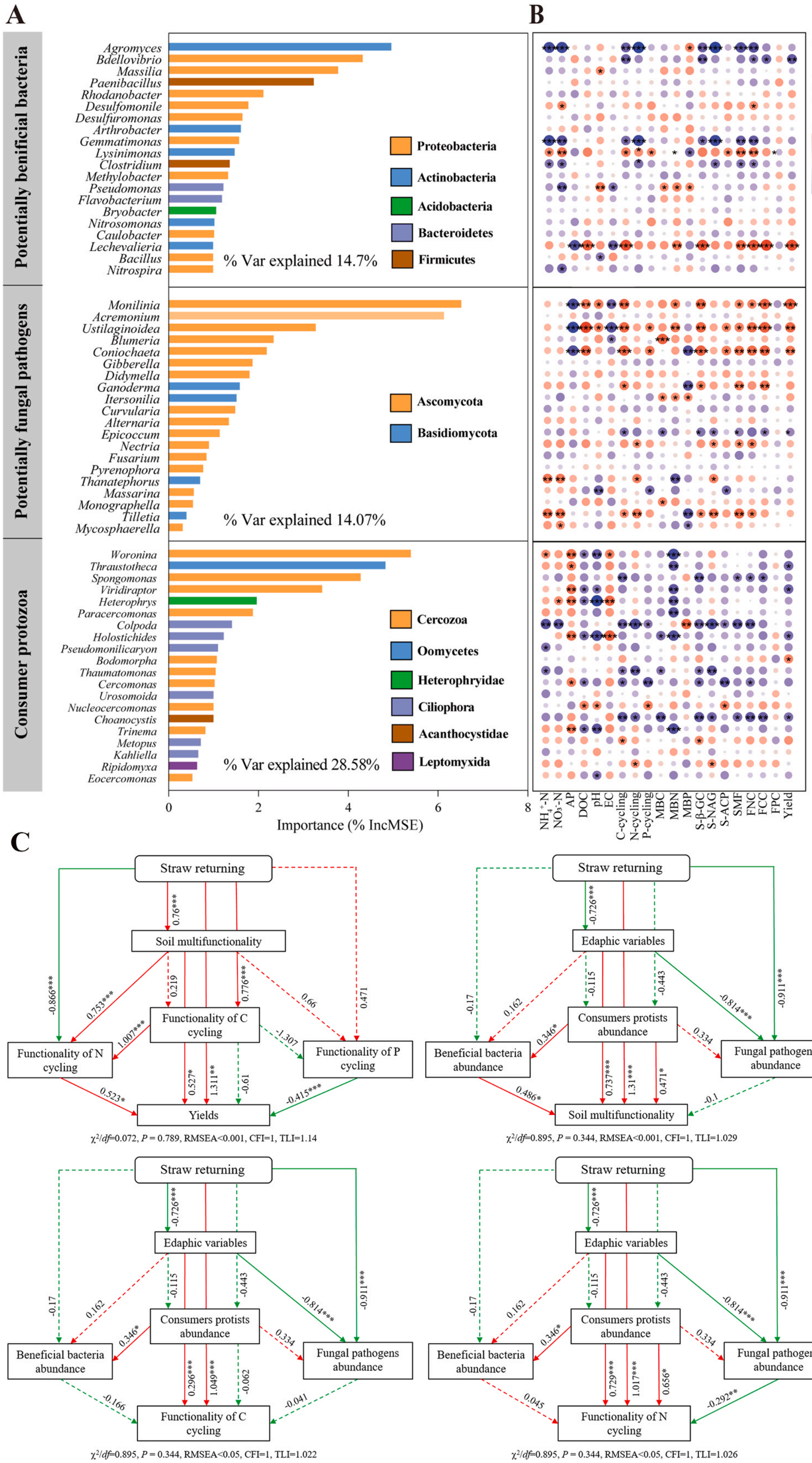

**Fig. 7.** Random forest model shows the top 20 most important genera of soil beneficial bacteria, fungal pathogens and consumer protists as key drivers of wheat yields, respectively (A). The bubble chart illustrates the correlations between key microbial taxa and wheat yield, as well as various soil physicochemical and biological properties (B). SEMs analyzing the effect of the straw returning on soil properties and microbiota (C). **$p < 0.01$, *$p < 0.05$.

### *4.2. Straw return amendments regulate soil functional microbiomes*

This study found that straw return affected microbial communities in certain ecological zones, particularly bacteria in leaves and fungi in rhizosphere soil. Straw return supplied an important external carbon source, greatly enhancing soil productivity and delivering crucial nutrients for the growth of soil microbes (Chen et al., 2023). The present research also identified a positive correlation between the DOC content in the soil and the diversity of rhizosphere fungi and bacteria. Specifically, for fungal communities, the return of both rice and rice–wheat straw resulted in significant increases in fungal niche breadth and α-diversity, likely due to reduced competition for carbon and nitrogen among fungi following straw return (Cong et al., 2020). Moreover, the R and RW treatments decreased the overall abundance of pathogenic fungi in the rhizosphere soil. Our findings substantiate the concept that the practice of straw incorporation can be considered an effective organic agricultural strategy for mitigating the incidence of soil-borne diseases in crops (Shan et al., 2021; Yu et al., 2023). Nevertheless, our result also showed that the long-term straw return has been associated with an increase in the abundance of pathogenic fungi in plants, notably *Mycosphaerella*, a pathogen responsible for causing wheat leaf spot disease. This observation aligns with prior research indicating that straw return leads to an increase in pathogenic fungi (Zhou et al., 2024). Previous research has demonstrated that higher rates of straw return create an environment conducive to the proliferation of pathogenic microorganisms, potentially contributing to plant diseases such as sharp eyespot and common rot in wheat (Ninkuu et al., 2025; Shan et al., 2021). Although our studies have demonstrated the suppression of pathogenic fungi in soil by straw incorporation, the complex mechanisms and risk assessment associated with pathogen accumulation in plants remain unexplored. Additional empirical field data on the biology and epidemiology of diseases are required. Therefore, it is essential to examine the long-term effects of soil management through controlled experiments, both on small and large scales, to address plant disease issues comprehensively.

Our result indicated that the R and RW treatments decreased the abundance of beneficial bacteria in wheat, including potential beneficial bacteria such as *Pseudomonas*, *Arthrobacter*, *Sphingomonas*, and *Bradyrhizobium*, which promote carbon cycling. Functional microorganisms (particularly *Pseudomonas*) play a crucial role in straw decomposition (Zhang et al., 2025). Our study primarily focused on the wheat maturity stage, by which time the straw had largely been completely decomposed, releasing substantial nutrients that provided abundant nitrogen and carbon sources for other microbial species. This likely led to niche competition that displaced these functional microorganisms (Liu et al., 2010; Zhang et al., 2022b). Moreover, functional redundancy exists in microbial communities, meaning that these communities can perform similar ecological roles even when certain microbial populations decline (Fetzer et al., 2015; Galand et al., 2018). In our study, this was evidenced by the fact that the reduced abundance of these microorganisms did not lead to decreased functional gene abundance in the soil (Galand et al., 2018; Louca et al., 2018). The RW treatment markedly decreased the abundance of soil predatory protozoa, a phenomenon that was likely caused by the anaerobic microenvironment under substantial straw incorporation. This environment accelerated the fermentation process, thereby suppressing protozoan metabolic activity (Zhang et al., 2024b). Overall, the results showed that straw return changed the microbial function and abundance of rhizosphere soil.

According to Mantel's test findings, the soil properties were important factors affecting the diversity of functional microorganisms (Fig. 6B–E). In this study, most environmental factors were positively correlated with fungal diversity, but they exerted a negative impact on bacteria, possibly because long-term straw return fostered a more homogeneous bacterial community in the field, with nutrient inputs promoting the growth of dominant species that occupied ecological niches (Fig. 3B), making the community more consistent. Most environmental factors were not significantly correlated with protozoa, consistent with Hu's findings that protozoan communities exhibited increased resilience to observed environmental changes (Hu et al., 2024b). Furthermore, negative correlations were observed between beneficial bacteria, consumer protozoa, and pathogenic fungi (Fig. 6A). These patterns suggest that beneficial bacteria and consumer protozoa exert a synergistic inhibitory effect on pathogen proliferation, aligning with earlier studies (Khan et al., 2016; Yan et al., 2024a). This interaction may explain the reduced abundance of beneficial bacterial and increased pathogenic fungal colonization in wheat plants observed in our study. Altogether, our findings suggest that the changes in rhizosphere soil physicochemical properties following straw incorporation represent a key driver of functional microbial community restructuring.

### *4.3. Key factors enhancing SMF under long-term straw return*

The positive impact of straw returning on ecosystem functions led to a significant increase in SMF, which is strongly correlated with particular functional soil microbes (Han et al., 2022). Moreover, shifts in the abundance of functional microbes significantly influence SMF (Wang et al., 2022). Returning straw enhanced the availability of key nutrients, which stimulated the proliferation and metabolic functions of certain soil microbial communities. This, in turn, activated the secretion of diverse enzymes that facilitated the decomposition of organic matter (Liu et al., 2023b). Straw return resulted in elevated levels of MBC and MBN, which positively influenced the abundance of genes associated with carbon and nitrogen cycling functions (Oliverio et al., 2020; Treseder et al., 2018). This observation may elucidate how the practice of straw returning contributes to the enhancement of soil carbon and nitrogen cycling functionality. We also found that these physicochemical factors were associated with changes in functional genes (Fig. 2C). This demonstrated a strong positive correlation between the increased levels of readily available carbon and nitrogen in soil and SMF following long-term straw return.

Random forest analysis identified potentially beneficial bacteria, pathogenic fungi, and consumer protists as significant predictors of yield variation (Fig. 7A). These findings further suggest that certain genera—such as *Agromyces*, *Arthrobacter*, and *Pseudomonas*, which are recognized for their beneficial roles—act as key contributors to SMF. Studies have shown that *Pseudomonas*, *Agromyces*, and *Arthrobacter* are involved in C cycling (Li et al., 2025; Zhang et al., 2024c), while the potentially fungal pathogenic genera *Alternaria*, *Epicoccum*, and *Mycosphaerella* are also dominant predictors of SMF. Although the potentially beneficial bacteria ranked highest in abundance declined after straw return, the abundance of all non-dominant species increased, including that of *Paenibacillus* and *Pantoea*. Studies have shown that many of these microorganisms are associated with straw decomposition and carbon and nitrogen cycling (Bao et al., 2020). Due to the functional redundancy of microorganisms, these microbial functions may have replaced those of conventional bacteria, thereby reducing the abundance of numerous functional bacterial groups without altering soil functionality (Galand et al., 2018; Louca et al., 2018). The heterogeneity of the soil environment is considered an important driver of the generation and maintenance of highly diverse microbial communities (Fierer, 2017). The majority of these microbes, which possess potential functional roles, demonstrate strong associations with factors such as soil pH, DOC, $NO_3^-$-N, and $NH_4^+$-N. Predatory, or consumer protists prey on bacteria and fungi (Geisen et al., 2018), thereby influencing ecosystem processes, including pathogen regulation and nutrient cycling (Sun et al., 2021). Our findings revealed that in acidic soils, the contents of DOC, pH, MBN, and MBC were inversely correlated with those of protists, underscoring the critical role of soil characteristics in shaping and regulating protist community dynamics within the soil ecosystem. Furthermore, our SEM demonstrated that physicochemical factors exert significant effects on rhizosphere microbial communities (Fig. 7C). SEM analysis offers further evidence that the prolonged application of straw return directly

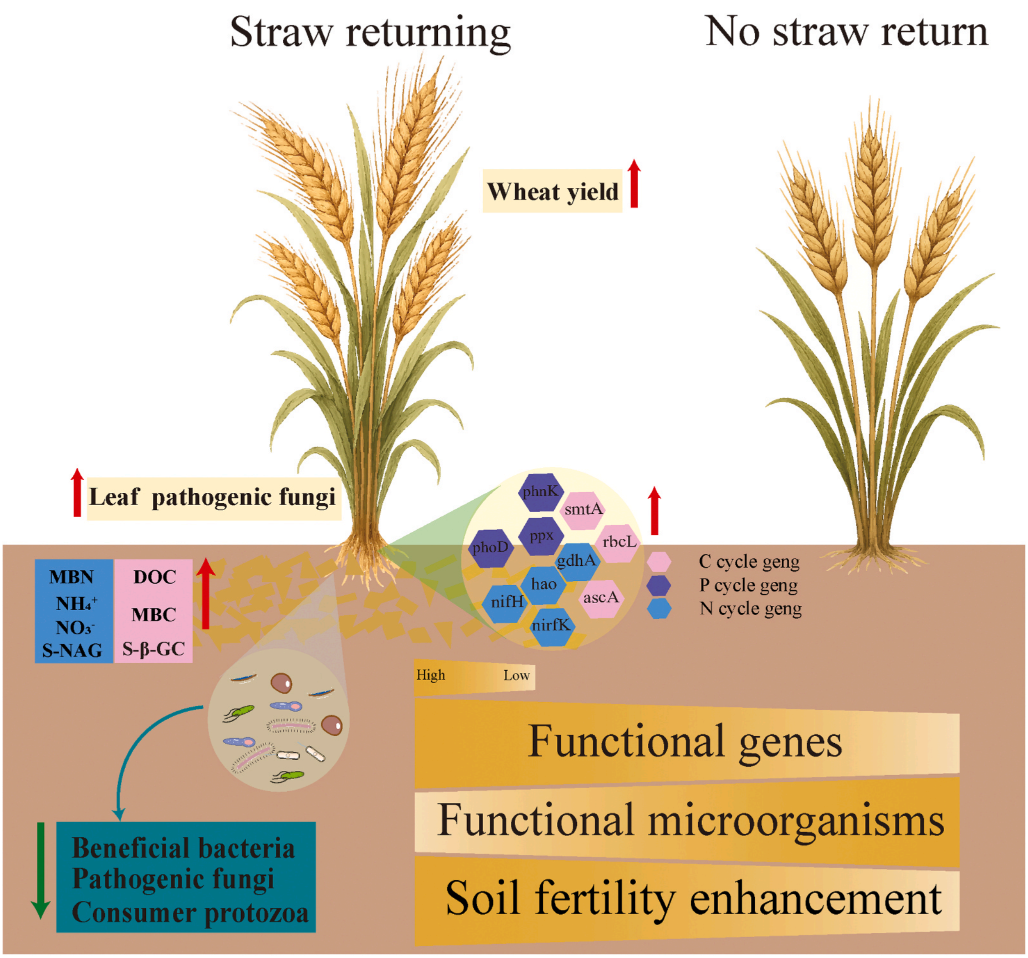

**Fig. 8.** Schematic model for the changes of rhizosphere microenvironment under straw returning.

enhances soil fertility, improves the soil environment, and promotes soil nutrient cycling functions. Additionally, it indirectly impacts nutrient cycling by reorganizing functional microbial communities through alterations in these physicochemical properties.

## 5. Conclusion

This study illustrates that the long-term application of straw return markedly improves multiple ecosystem functions, including crop productivity (particularly in the RW treatment, which exhibited an 8.07 % increase compared to the non-return treatment), soil nutrient retention, and soil enzyme activity. The prolonged practice of straw return significantly elevated the abundance of functional genes associated with carbon and nitrogen cycles to varying extents (Fig. 8). Furthermore, straw return increased the abundance of potentially pathogenic fungi in leaves while reducing their presence in rhizosphere soil. Microbial communities involved in carbon and nitrogen cycles demonstrated statistically significant correlations with soil multifunctionality, primarily influenced by soil pH and nutrient availability, particularly nitrogen and carbon. The results indicate that the practice of straw return leads to changes in the diversity and abundance of functional microbial communities, thereby facilitating nutrient cycling and enhancing soil multifunctionality. Specifically, the incorporation of straw from double-cropping systems significantly modifies the structure and function of soil microbial communities. These modifications enhance soil nutrient cycling processes, which ultimately contribute to improved crop yields. This study provides critical theoretical insights and empirical evidence in support of the wider adoption of straw return practices.

## CRediT authorship contribution statement

**Jiahao Liu:** Methodology, Investigation, Formal analysis. **Zhixiang Han:** Investigation, Formal analysis. **Wenkang Yan:** Writing – original draft, Validation, Methodology, Formal analysis, Conceptualization. **Mingyang Xia:** Methodology, Investigation, Formal analysis. **Christopher Rensing:** Writing – review & editing. **Hend A. Alwathnani:** Writing – review & editing. **Zhong Li:** Writing – original draft, Resources, Conceptualization. **Hongmiao Wu:** Writing – review & editing, Supervision, Project administration, Formal analysis, Conceptualization. **Beibei Chen:** Investigation, Formal analysis. **Wenge Wu:** Writing – review & editing, Supervision, Project administration, Conceptualization.

## 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.2025.110015.

## Data availability

The data that has been used is confidential.

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