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Harnessing Microbial Agents to Improve Soil Health and Rice Yield Under Straw Return in Rice–Wheat Agroecosystems

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Abstract

We clarified the effect of wheat straw return combined with microbial agents on rice yield and soil properties. A field experiment was conducted using hybrid indica rice '*Chuankangyou 2115*' and five treatments: no wheat straw return (T_1), wheat straw return alone (T_2), T_2+ microbial agent application (*Bacillus subtilis*/*Trichoderma harzianum* = 1:1) (T_3); T_2+ microbial agent application (*Bacillus subtilis*/*Trichoderma harzianum* = 3:1) (T_4); T_2+ microbial agent application (*Bacillus subtilis*/*Trichoderma harzianum* = 1:3) (T_5). T_2–T_5 significantly increased dry matter accumulation, soil total N, ammonium N, nitrate N, and organic matter, improving yield by 3.81–26.63%. T_3 exhibited the highest yield increases in two consecutive years. At the jointing and heading stages, *Penicillium* and *Saitozyma* dominated under T_3 and positively correlated with dry matter, yield, and nitrogen levels. Straw return combined with *Bacillus subtilis* and *Trichoderma harzianum* (20 g m^{-2} each) enhanced soil nitrogen availability and dry matter accumulation and translocation. Our findings guide efficient straw utilization, soil microbial regulation, and sustainable high-yield rice production.

Keywords: rice; wheat straw return; microbial agents; soil nutrients; soil fungi

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1. Introduction

The rice–wheat rotation system is one of the largest agricultural production systems in the world and is primarily distributed in the Yangtze River Basin in China, where it accounts for 72% of the total food production [1]. However, this high-yield farming system generates a large amount of straw residue, producing approximately 4500 kg hm^{-2} of wheat straw and approximately 9000 kg hm^{-2} of rice straw annually [2]. The main chemical components of wheat straw are cellulose, hemicellulose, lignin, and a small amount of inorganic substances. Wheat straw return can reasonably utilize resources, improve soil structure, and increase soil organic matter, providing nutrients such as nitrogen (N), phosphorus (P), potassium (K), and trace elements to the soil, significantly enhancing land productivity and improving soil structure [3]. However, direct straw return is associated with issues such as a long decomposition period and slow nutrient release [4]; after 124 days of incubation, wheat straw achieved a carbon (C) release rate of only 66.58%, a nitrogen (N) release rate of 49.26%, and a phosphorus (P) release rate of 59.93%. Additionally, incomplete straw decomposition can lead to the proliferation of pathogens and pests, increasing the risk of soil-borne diseases, and consequently reducing crop yields [5]. Therefore, reducing

the adverse effects of straw decomposition, while ensuring straw return, remains a focal point in current agricultural production.

Previous studies have shown that soil microorganisms play a crucial role in the decomposition of straw. Soil microorganisms break down complex organic substances in straw, such as cellulose, hemicellulose and lignin, into simpler compounds by secreting enzymes and other bioactive substances. During the process of straw decomposition, bacteria have an advantage in breaking down simple organic compounds, while fungi play a significant role in decomposing more complex lignin and cellulose [6]. This process not only accelerates the release of nutrients but also increases the content of soil organic matter, thereby significantly enhancing soil fertility. Microbial agents, including enzymes, microbial cells, and their metabolic products, have been widely used in agricultural production to support the modern agricultural push for "green" and "ecological" practices. Enzymes such as ligninolytic enzymes (e.g., lignin peroxidase, manganese peroxidase, and laccase) play a crucial role in degrading complex organic compounds in agricultural residues, making nutrients more available to plants and improving soil structure [7]. Microbial cells, particularly plant growth-promoting rhizobacteria (PGPR) and mycorrhizal fungi, enhance soil fertility and plant health by improving nutrient uptake, producing plant growth hormones, and suppressing soil-borne pathogens [8]. Additionally, metabolic products from microorganisms, such as bioactive compounds and organic acids, can further improve soil health and promote beneficial microbial interactions [9]. Various microbial agents, including bacteria and fungi, have been used to improve agricultural practices. *Bacillus subtilis* and *Trichoderma harzianum* are commonly used in agricultural applications. *Bacillus subtilis* is a common rhizosphere bacterium in soil that plays a key role in providing plants with resistance to biotic and abiotic stresses through mechanisms such as induction of systemic resistance (ISR), biofilm formation, and lipopeptide production. Moreover, *Bacillus* spp. can purify metal-contaminated soil [10], and *Bacillus subtilis* inoculation significantly reduces the bioavailability of soil Pb through bioadsorption, bioaccumulation, and conversion into more stable forms [11]. *Bacillus subtilis* also promotes nutrient cycling by increasing nitrogen fixation, phosphorus solubilization, and potassium dissolution, thereby improving fertilizer utilization efficiency [12]. de Lima et al. [13] demonstrated that under water-limited conditions, inoculation of *Bacillus subtilis* in maize and soybean increased leaf water content and reduced leaf antioxidant activity to cope with water stress. Inoculation with *Trichoderma harzianum* significantly increases seed germination and seedling growth rates in wheat, carrot, and potato, and enhances plant dry weight [14]; however, excessive concentrations can lead to inhibitory effects [15]. Oliveira et al. [16] found that *Trichoderma harzianum* increased root length, surface area, and total root dry weight by 17.0%, and improved P content (34.0%) and total dry matter (22.0%) in plants fertilized with soluble phosphate. Al-Zuhairi et al. [17] showed that a well-developed root system can enhance nutrient absorption, thereby regulating plant growth and development. Recent studies have suggested that *Trichoderma harzianum* enhances plant resistance to abiotic stress via plant interactions [18]. Cocoa seedlings treated with *Trichoderma harzianum* show rapid growth and strong drought tolerance under unfavorable conditions. Additionally, *Trichoderma harzianum* is a beneficial fungus that regulates plant growth and development by combating soil-borne pathogens that infect plant roots [19]. Previous studies have extensively reported the functional mechanisms of single inoculant treatments with *Bacillus subtilis* and *Trichoderma harzianum*. However, there is limited research on the combination of these two microbial agents for wheat straw decomposition, especially concerning their effects on the paddy soil environment. We hypothesize that the combined application of *Bacillus subtilis* and *Trichoderma harzianum* will synergistically promote straw decomposition, improve soil nitrogen availability, and enhance rice yield under straw-return conditions.

Therefore, this study aimed to investigate the effects of the combined application of *Bacillus subtilis* and *Trichoderma harzianum* on the decomposition of wheat straw in paddy soil (the texture of paddy soil is mostly loam, containing clay, silt, and sand particles. This type of soil combines the advantages of both clay and sandy soils, exhibiting strong cohesiveness and water retention capacity [20]) and subsequent rice yield. We also elucidate the intrinsic synergistic interactions between microbes and the paddy soil environment. These findings may guide efficient straw utilization, environmental pollution reduction, and rice yield enhancement in rice–wheat rotation systems.

2. Materials and Methods

2.1. Experimental Site and Materials

This study was conducted in 2022 and 2023 at the Wenjiang Experimental Station of Sichuan Agricultural University (Longitude: 103.873794; Latitude: 30.716057). The rice variety tested was *Chuankangyou 2115*, a three-line indica hybrid rice developed by Sichuan Agricultural University, the Crop Research Institute of Sichuan Academy of Agricultural Sciences, and Sichuan Lüdan Zhicheng Seed Industry Co., Ltd. (Chengdu, China), with a total growth duration of 151.8 days. The microbial agents *Bacillus subtilis* and *Trichoderma harzianum* were provided by Sichuan Green Microbial Technology Co., Ltd. (Chengdu, China), at a concentration of 210^{10} CFU g^{-1}. After the preceding wheat crop was harvested, the wheat straw was chopped into pieces of 5–10 cm and returned to the field at a rate of 4710 kg·hm^{-2} (with a moisture content of 10%). The plowed layer of the experimental field consisted of sandy loam soil, classified according to the World Reference Base for Soil Resources.

The baseline soil fertility in 2022 was as follows: total N 1.96 g kg^{-1}, organic matter 18.05 g kg^{-1}, alkali-hydrolyzed N 106.23 mg kg^{-1}, available P 25.11 mg kg^{-1}, and available K 85.23 mg kg^{-1}. In 2023, the baseline fertility was as follows: total N 2.26 g kg^{-1}, organic matter 20.45 g kg^{-1}, alkali-hydrolyzed N 115.82 mg kg^{-1}, available P 28.19 mg kg^{-1}, and available K 87.88 mg kg^{-1}.

2.2. Model of the Experiment

In both years, a single-factor completely randomized design was adopted. The microbial agent ratios and application rates were determined with reference to the method described by Lu et al. [21]. The treatments were as follows (Table 1): ① No straw return (T_1); ② Straw return (T_2); ③ Straw return + (*Bacillus subtilis*: *Trichoderma harzianum* = 1:1) (T_3); ④ Straw return + (*Bacillus subtilis*: *Trichoderma harzianum* = 3:1) (T_4); ⑤ Straw return + (*Bacillus subtilis*: *Trichoderma harzianum* = 1:3) (T_5). A compound fertilizer (N-P-K, 20-8-12) was applied to supply a total of 150 kg hm^{-2} of nitrogen, distributed at a ratio of 3:3:4 across the basal, tillering, and panicle stages, respectively. Each treatment was replicated three times, and the area of each plot was 4 × 5 m = 20 m^2. In both years, rice seedlings were sown on 15 April, wheat straw was incorporated into the soil on 15 May, the seed transplantation sites were chosen randomly within each experimental plot to ensure uniformity, manual transplanting was performed on 15 May, and harvesting was performed on 10 September. Plant spacing was 33.3 cm × 16.7 cm, with a single seedling transplanted per hill.

Microbial agents were applied two times, i.e., 1 day and 30 days after transplantation (16 May and 16 June), using a dilution of 1 g of agents per 10 cm^3 of distilled water, and sprayed evenly. Each application used 40 g m^{-2} of the agents. Dikes (40 cm wide) were constructed between the plots and covered with plastic film to enable precise water and fertilizer management. In addition, we applied bisultap and pretilachlor at the recommended dose 7 days before or after the microbial agent treatment to manage the diseases, pests

and weeds of rice while avoiding direct interaction or antagonism with *Bacillus subtilis* and *Trichoderma harzianum*.

Table 1. Experimental design variants and descriptions.

Treatment	Straw Return	Microbial Agents (*Bacillus subtilis*/*Trichoderma harzianum*)
T_1	None	None
T_2	Straw return	None
T_3	Straw return	*Bacillus subtilis*/*Trichoderma harzianum* = 1:1
T_4	Straw return	*Bacillus subtilis*/*Trichoderma harzianum* = 3:1
T_5	Straw return	*Bacillus subtilis*/*Trichoderma harzianum* = 1:3

2.3. Parameters and Measurement Techniques

2.3.1. Soil Nutrients

Soil samples (0–20 cm depth) were collected at the rice tillering, jointing, heading, and maturity stages using a five-point sampling method. Specifically, five sampling points were randomly selected within each experimental plot to ensure representativeness. After air drying under natural conditions, the samples were ground and sieved through a 120-mesh screen. Soil total nitrogen (TN) was determined using the Kjeldahl method. Organic matter (OM), nitrate nitrogen (NN) and ammonium nitrogen (AN) were determined according to Bao [22]. Briefly, organic matter was measured using potassium dichromate oxidation—soil samples were treated with a mixture of potassium dichromate and sulfuric acid, followed by heating and titration with ferrous ammonium sulfate to determine the organic carbon content, which was then converted to organic matter content; nitrate N was measured using UV spectrophotometry—soil extracts were prepared with 2 M KCl, and nitrate concentration was determined at a wavelength of 210 nm; ammonium N was measured using the indophenol blue colorimetric method—soil extracts were prepared with 2 M KCl, and the ammonium concentration was determined by reacting with salicylate and hypochlorite, followed by colorimetric analysis at 625 nm.

2.3.2. Soil Fungal Community Structure

At the rice jointing and heading stages, 5.0 g of fresh soil surrounding the rice roots was collected and placed in sterile EP tubes. Samples were immediately frozen in liquid nitrogen and stored at −80 °C. Soil DNA was extracted using a Soil DNA Kit (Omega Bio-tek, Inc., Norcross, GA, USA). Fungal ITS_V1 regions were amplified and sequenced by Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China) using the following primers: forward primer F: GGAAGTAAAAGTCGTAACAAG and reverse primer R: GCTGCGTTCTTCATCGATG. The resulting fungal sequences were aligned to the UNITE database for taxonomic identification.

2.3.3. Dry Matter Accumulation and Yield Determination

At rice jointing, heading, and maturity stages, five rice plants per plot were sampled based on the average number of tillers. The subsequent processing of the samples was carried out in accordance with the method of Wang et al. [23]. Plants were separated into culms, sheaths, leaves, and spikes. The samples were first heated at 105 °C for 30 min to inactivate the enzymes, and then dried at 80 °C to a constant weight. At harvest, the border rows of each plot were excluded, and grain yield was measured based on the number of harvested plants and adjusted to a standard moisture content of 13.5%.

2.4. Calculation of Physiological Indices

Leaf Matter Transport Rate (*MTL*, %):

$$MTL = \frac{Dry\ weight\ (DW)\ of\ leaves\ at\ headin - DW\ of\ leaves\ at\ maturity}{DW\ of\ leaves\ at\ heading} \times 100\% \quad (1)$$

Matter Contribution Rate of Leaf (*MCL*, %):

$$MCL = \frac{DW\ of\ leaves\ at\ heading - DW\ of\ leaves\ at\ maturity}{DW\ of\ grains} \times 100\% \quad (2)$$

Matter Transport Rate of Stem Sheath (*MTSS*, %):

$$MTSS = \frac{DW\ of\ leaves\ at\ heading - DW\ of\ leaves\ at\ maturity}{DW\ of\ stem\ sheath\ at\ heading} \times 100\% \quad (3)$$

Matter Contribution Rate of Stem Sheath (*MCSS*, %):

$$MCSS = \frac{DW\ of\ stem\ sheath\ at\ heading - DW\ of\ stem\ sheath\ at\ maturity}{DW\ of\ grains} \times 100\% \quad (4)$$

Dry Matter Accumulation at Each Growth Stage (*ADM*, kg hm^{-2}):

$$ADM = W2 - W1 \quad (5)$$

Growth Rate of Population at Each Growth Stage (*GRP*, g $m^{-2}d^{-1}$):

$$GRP = \frac{W2 - W1}{t2 - t1} \quad (6)$$

where $W1$ and $W2$ represent the dry matter accumulation at two successive measurements and $t1$ and $t2$ are the time intervals between the two measurements.

2.5. Statistical Analysis of Results

The data were organized using Microsoft Excel 2019, and graphs were prepared using Origin 2023. Statistical analyses were performed using SPSS version 24.0. Differences between treatment means were evaluated using the Least Significant Difference (LSD) test at a significance level of $\alpha = 0.05$.

3. Result

3.1. Rice Yield and Dry Matter Accumulation

All treatments affected rice yield and dry matter accumulation during critical growth stages (LSD test, $\alpha = 0.05$), with consistent trends across both experimental years (Treatment × Year interaction $p > 0.05$) (Table 2). Compared to T_1, grain yields increased by the following amounts: T_2: 3.81% and 9.14%; T_3: 17.15% and 21.02%; T_4: 10.63% and 12.19%; T_5: 10.38% and 10.86%, with the T_3 treatment consistently showing significantly higher yields than the other treatments. The F values from the analysis of variance further underscore the significance of these differences. For instance, the F values for grain yield in 2022 and 2023 were 18.16 and 60.88, respectively, indicating highly significant differences among the treatments. At the heading stage, the T_3 treatment showed the highest stem sheath and leaf dry weights (stem sheath: 1.2–17.1% higher than $T_1/T_2/T_4/T_5$ (minimum: 2023 T_3 vs. T_4; maximum: 2022 T_3 vs. T_1); leaf: 9.2–47.8% higher than $T_1/T_2/T_4/T_5$ (minimum: 2023 T_3 vs. T_4; maximum: 2023 T_3 vs. T_1)). The leaf and spike dry weights under T_3 were significantly higher than those under other treatments at the maturity stage

(leaf dry weight: 8.61–35.89% higher than $T_1/T_2/T_4/T_5$ (minimum: 2023 T_3 vs. T_4 [+8.61%]; maximum: 2023 T_3 vs. T_1 [+35.89%]); spike dry weight: 2.80–22.65% higher (minimum: 2023 T_3 vs. T_4 [+2.80%]; maximum: 2022 T_3 vs. T_1 [+22.65%])). T_3 exhibited strong advantages in stem sheath and leaf dry matter accumulation at the heading stage, and in spike dry weight at maturity, with particularly more significant increases compared to the other treatments in 2022.

Table 2. Effects of straw return and combined microbial agents application on selected indices of rice yield.

Year	Treatment	Grain Yield (kg hm^{-2})	Heading Stage			Maturing Stage		
			Stem (kg hm^{-2})	Leaf (kg hm^{-2})	Spike (kg hm^{-2})	Stem (kg hm^{-2})	Leaf (kg hm^{-2})	Spike (kg hm^{-2})
2022	T_1	9270.37 c	6603.60 d	2877.00 d	3175.80 b	4495.27 b	2064.80 c	9788.80 d
	T_2	9623.47 c	6809.87 c	3530.40 c	3397.20 a	4557.00 b	2452.80 b	10,902.60 c
	T_3	10,860.02 a	7733.27 a	4027.53 a	3230.40 b	4897.00 a	2691.07 a	12,353.13 a
	T_4	10,256.13 b	7209.53 b	3655.47 b	3304.93 ab	4804.67 a	2477.80 b	11,532.00 b
	T_5	10,232.76 b	7284.00 b	3516.20 c	3240.93 b	4828.13 a	2439.00 b	10,659.93 c
	F value	18.16 **	55.24 **	51.99 **	3.75 *	13.76 **	45.82 **	53.35 **
2023	T_1	9482.05 c	6837.07 c	2595.47 d	3214.13 b	4735.13 a	2055.67 c	9190.87 c
	T_2	10,436.26 b	6856.20 c	2980.87 c	3353.87 a	4737.60 a	2283.60 c	9336.87 c
	T_3	11,474.18 a	7850.20 a	3836.87 a	3139.60 b	4843.33 a	2793.60 a	11,272.80 a
	T_4	10,638.25 b	7755.93 a	3356.47 b	3332.33 b	4825.33 a	2554.87 b	10,965.60 a
	T_5	10,511.69 b	7250.20 b	3220.20 b	3198.60 a	4874.07 a	2499.00 b	10,023.23 b
	F value	60.88 **	34.39 **	59.14 **	8.29 **	1.19	38.34 **	55.83 **

Note: The stems, leaf and spike all represent their respective dry weights. Data in the same column are marked with different letters indicating significant differences at the 5% level; *, ** indicate significance at the 0.05 and 0.01 levels, respectively. Columns with the same letters (e.g., all values marked with 'a') indicate that there are no significant differences among those values at the 5% level.

3.2. Dry Matter Transport and Crop Growth During Grain Filling

Different treatments influenced the material transport characteristics and population growth rate of rice, showing consistent trends throughout the two-year experiment (Table 3). Compared with T_1, the T_2–T_5 treatments significantly increased the material transport rate of leaves (MTL) by 7.74–30.78%; for material transport rate of stems and sheaths (MTSS), compared with T_1, microbial agent treatment (T_3–T_5) significantly increased MTSS by 4.51–23.86% ($p < 0.05$). However, T_2 showed mixed effects: a non-significant increase in 2022 but a slight decrease in 2023. This highlights the critical role of microbial agents in consistently enhancing stem sheath transport efficiency. T_3 demonstrated a marked advantage in all transport-related parameters. Specifically, MTL and MCL (material contribution rate of leaves) under T_3 increased by 17.54–30.78% and 30.60–57.75%, respectively, relative to T_1, while MTSS rose by 14.88–23.86%. Compared with the uninoculated treatment (T_1–T_2), the combined application of microbial agents (T_3–T_5) increased dry matter accumulation (ADM) to varying degrees, with T_3 performing the best over two years. Although there were annual variations (for example, T_2 was greater than T_5 in 2022), the enhancing effect of microbial agents was relatively strong in both years. The population growth rate (GRP) at various growth stages exhibited a trend similar to that of ADM, and T_3 showed the highest performance in both years, increasing by 34.04% and 23.17%, respectively, compared to T_1. As shown in Table 3, the material contribution rate of stems and sheaths (MCSS) in 2022 was significantly higher under T_3 and T_5 treatments, with increases of 6.59–6.96% over T_1. In 2023, peak MCSS values were observed under T_3 and T_4, increasing by 15.64–15.82% relative to T_1. Notably, the T_3 treatment stood out in terms of synergistically improving both material transport efficiency and population growth. In 2023, the MTL and GRP under T_3 were significantly higher, increasing by 17.53–30.78% and 23.17–34.04%, respectively, compared to those under the other treatments. These findings suggest that the T_3 treatment

significantly enhanced the translocation of dry matter to the spikes by improving MTSS and MTL, ultimately contributing to the formation of a high-yield population structure.

Table 3. Effects of straw return and combined application of microbial agents on selected indices of material transport and rice population growth during the grain filling stage.

Year	Treatment	MTL (%)	MCL (%)	MTSS (%)	MCSS (%)	ADM (kg hm^{-2})	GRP (g m^{-2} d^{-1})
2022	T_1	28.22 b	8.30 b	31.92 b	21.54 b	3692.47 c	12.31 c
	T_2	30.51 ab	9.89 a	33.08 b	20.67 b	4174.93 bc	13.92 bc
	T_3	33.17 a	10.84 a	36.67 a	22.96 a	4950.00 a	16.50 a
	T_4	32.22 a	10.21 a	33.36 b	20.85 b	4644.53 ab	15.48 ab
	T_5	30.61 ab	10.11 a	33.71 b	23.04 a	3885.93 c	12.95 c
	F value	4.99 *	4.79 *	9.88 **	6.77 **	11.39 **	11.41 **
2023	T_1	20.79 c	5.87 c	30.92 b	23.07 b	3315.87 b	11.05 b
	T_2	23.39 bc	7.49 b	30.72 b	22.54 b	3186.27 b	10.62 b
	T_3	27.19 a	9.26 a	38.30 a	26.68 a	4083.07 a	13.61 a
	T_4	23.85 b	7.31 b	37.78 a	26.72 a	3901.07 a	13.00 a
	T_5	22.40 bc	7.20 b	32.77 b	23.70 b	3727.29 ab	12.43 ab
	F value	7.95 **	12.09 **	32.92 **	8.40 **	5.04 *	5.05 *

Note: The meanings of the letters and asterisks marked in this table are the same as those in Table 2. MTL: Material transport rate of leaves; MCL: Material contribution rate of leaves; MTSS: Material transport rate of stem sheath; MCSS: Material contribution rate of stem sheath; ACM: Accumulation of dry matter; GRP: Growth rate of the population; *, ** indicate significance at the 0.05 and 0.01 levels, respectively.

In addition, as shown in Table 3, during the two years, the F values of indicators such as MTL, MTSS, ADM and GRP all reached significant ($p < 0.05$) or extremely significant ($p < 0.01$) levels, indicating that the differences among treatments were statistically significant. It further supports the positive effect of microbial agents combined with straw returning to the field on improving the efficiency of material transport and the growth rate of the population.

3.3. Chemical Characteristics of Soil

During the critical rice growth stages, different treatments significantly influenced the soil total N, ammonium N, nitrate N, and organic matter content ($p < 0.05$). T_3 and T_5 exhibited the strongest effects, though their impact was highlighted by different indicators (Figure 1). T_3 treatment consistently maintained high levels of total soil N and ammonium N (Figure 1A,B). At both the tillering and jointing stages, T_3 significantly increased total soil nitrogen content compared to T_1 and T_2, demonstrating its stability in promoting early-stage nitrogen accumulation. Nitrate N content exhibited a dynamic pattern of 'increasing at the tillering stage but decreasing at the jointing stage' during the growth period (Figure 1C). Although T_3 consistently maintained the highest nitrate levels in 2022, T_5 showed a slight advantage in 2023.

Regarding soil organic matter content, the T_5 treatment was superior to other treatments, and the soil organic matter content at the jointing stage was significantly higher than that of T_1–T_4 (Figure 1D). Except for the jointing stage in 2023, T_5 maintained a high level of soil organic matter during other key growth periods, demonstrating a sustained ability to accumulate organic matter.

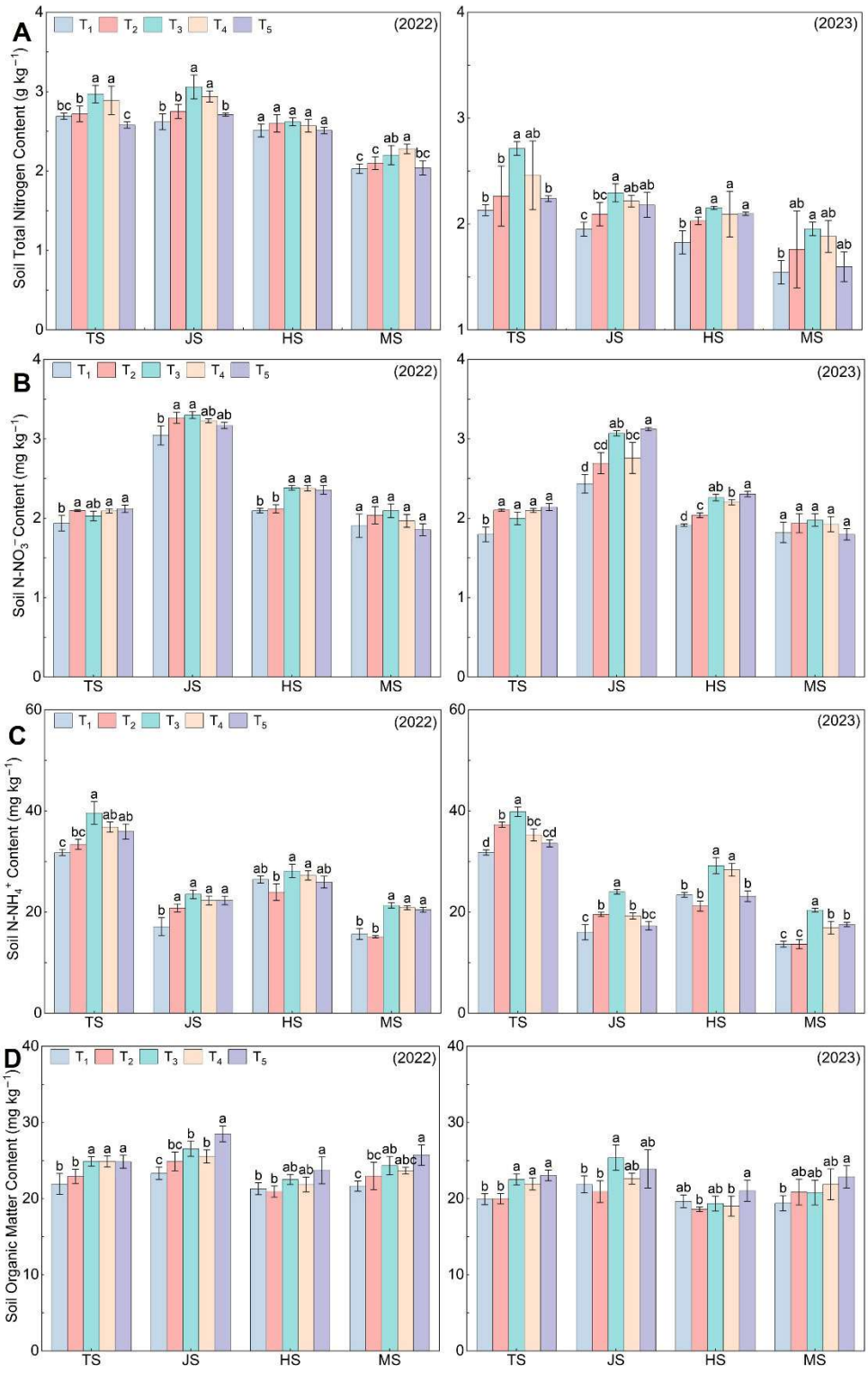


Figure 1. Effects of wheat straw return and combined microbial agent application on selected chemical parameters of soil. (**A**): Soil Total N; (**B**): nitrate N; (**C**): ammonium N; (**D**): organic matter. Note: The meanings of the letters marked in this figure are the same as those in Table 1. TS: Tillering stage; JS: Jointing stage; HS: Heading stage; MS: Maturity stage.

3.4. Fungal Microbial Composition

3.4.1. Rarefaction Curves

After the application of microbial agents, the analysis of soil fungal rarefaction curves demonstrated that the curves of all treatments gradually plateaued with increasing sequenc-

ing depth, indicating that the sequencing effort sufficiently captured the majority of species present in the samples, confirming that the sequencing quantity met the requirements of analysis (Figure 2).

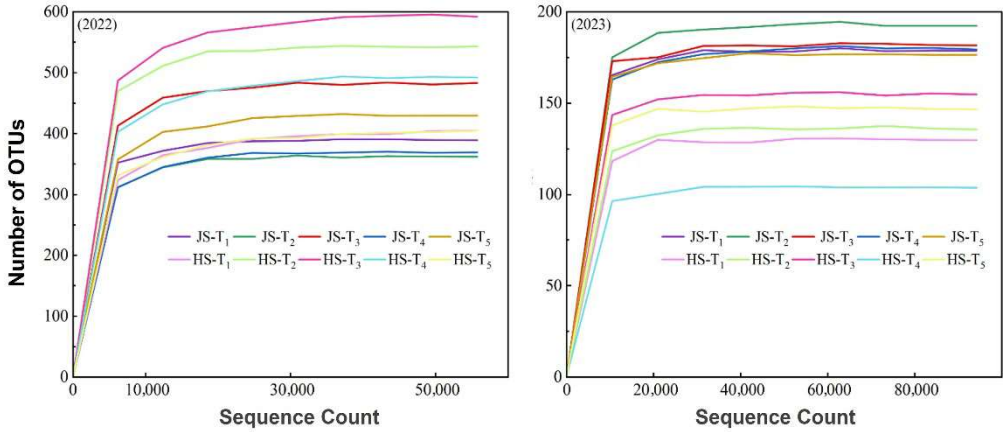


Figure 2. Dilution curves of soil fungi at harvest time with microbial inoculant treatment. Note: OTUs: Operational Taxonomic Units.

3.4.2. Soil Microbial Diversity Analysis

All treatments significantly influenced the diversity of the soil fungal communities. Over the two-year experiment, the Chao1 index and Shannon index exhibited consistent temporal patterns (Table 4). All the samples achieved sequencing coverage indices exceeding 90%, validating the reliability of the detection results. Significant *F*-values ($p < 0.01$) for Chao1 and Shannon indices across treatments confirm that microbial inoculants substantially altered fungal community diversity. Compared to other treatments, the T_3 treatment significantly enhanced fungal diversity during the critical growth stages. In 2022, at the jointing stage, T_3 recorded the highest Chao1 and Shannon indices, which were 25.03% and 2.95% higher than those of T_1, respectively. By the heading stage, the Chao1 index advantage of T3 increased to 46.15% over T_1. During the 2023 heading period, the Chao1 and Shannon indices of the T_3 treatment were the highest, with Chao1 being 49.32% higher than that of the worst-performing T_5 treatment. The Shannon index was 15.76% higher than that of the worst-performing T_2 treatment. Notably, although T_5 achieved the annual peak Shannon index at the jointing stage, its Chao1 index plummeted by 42.24% at the heading stage. Across the two years, T_3 consistently exhibited significantly higher Chao1 indices at the heading stage than the other treatments ($p < 0.01$). Furthermore, from the jointing to heading stages in 2022, T_3 demonstrated a 21.70% increase in the Chao1 index, which significantly exceeded those of T_4 and T_5. The T_2 treatment reached the peak Chao1 index at the jointing stage in 2023 but declined by 29.49% at the heading stage, indicating the limited efficacy of single straw return. In contrast, the T_3 treatment likely promoted soil nutrient cycling efficiency by maintaining higher Shannon and Chao1 indices, thereby contributing to enhanced rice yields.

Table 4. Soil fungal community diversity indices with microbial inoculant treatment.

Year	Treatment	Jointing Stage			Heading Stage		
		Chao1	Shannon	Coverage	Chao1	Shannon	Coverage
2022	T_1	389.16 c	5.43 ab	0.9999 a	405.17 d	4.63 c	1.00 a
	T_2	429.59 b	5.19 abc	0.9996 a	404.88 d	5.26 b	1.00 a
	T_3	486.53 a	5.59 a	0.9996 a	592.11 a	6.11 a	1.00 a
	T_4	362.07 c	5.01 bc	0.9997 a	543.55 b	5.22 b	1.00 a

Table 4. *Cont.*

Year	Treatment	Jointing Stage			Heading Stage		
		Chao1	Shannon	Coverage	Chao1	Shannon	Coverage
2023	T_5	369.23 c	4.85 c	0.9997 a	492.24 c	5.22 b	1.00 a
	F value	19.86 **	5.43 *	1.67	60.87 **	21.46 **	0.21
	T_1	178.80 b	4.26 c	1.00 a	129.75 b	3.94 bc	1.00 a
	T_2	192.37 a	4.73 a	1.00 a	135.63 b	3.87 c	1.00 a
	T_3	181.69 ab	4.55 b	1.00 a	154.84 a	4.48 a	1.00 a
	T_4	176.45 b	4.82 a	1.00 a	146.63 a	4.20 ab	1.00 a
	T_5	179.54 b	4.88 a	1.00 a	103.69 c	4.28 a	1.00 a
	F value	3.14	27.88 **	1.17	35.81 **	7.21 **	2.29

Note: The meanings of the letters and asterisks marked in this table are the same as those in Table 2. The diversity indices of soil fungi in Chao1, Shannon and coverage are shown in the table. Chao1 index is used to evaluate the richness of the community, where the higher the index value, the greater the richness of the community. The Shannon index was used to evaluate species diversity, and the higher the index value, the greater the community diversity. *, ** indicate significance at the 0.05 and 0.01 levels, respectively.

3.4.3. Soil Fungal Community Composition

Over the two-year experiment, the combined relative abundances of *Ascomycota* and *Basidiomycota* across all treatments exceeded 75% from the jointing to heading stages (Figure 3). In 2022, the fungal communities across treatments exhibited similar phylum-level relative abundance patterns at the jointing stage. However, at the heading stage, the relative abundance of *Basidiomycota* in T_3 and T_5 increased further and was significantly higher than that in T_1, T_2, and T_4. Additionally, a decline in the relative abundance of *Mortierellomycota* was observed from the jointing to heading stages across all treatments. The treatment effects observed in the 2023 experiment mirrored those observed in the 2022 experiment. However, notable shifts included the complete disappearance of *Glomeromycota* at heading stage in 2023 across all treatments, contrasting with its presence in 2022; the T_5 treatment exhibited a notably higher relative abundance of *Rozellomycota* at the jointing stage than the other treatments.

Different treatments significantly influenced the fungal community structure, with the relative abundances of key functional taxa, such as *Penicillium*, *Saitozyma*, and *Marquandomyces* exhibiting treatment-specific differences (Figure 4). The T_3 treatment consistently exhibited significant enrichment of *Penicillium* at the jointing stage across both years, with a markedly higher relative abundance than that in the other treatments. Furthermore, the relative abundance of *Saitozyma* in T_3 increased from the jointing to the heading stages during both years and remained significantly higher compared with the other treatments. During the jointing stage in 2023, *Penicillium*, *Marquandomyces*, and *Westerdyleiia* exhibited relatively higher abundances under the T_3 treatment, significantly surpassing those of the other treatments, whereas the T_1 and T_2 treatments displayed lower relative abundances. Compared to T_1, the T_2 treatment exhibited a significantly higher relative abundance of the fungal genus *Coniochaeta*. In summary, the T_3 treatment consistently maintained a higher relative abundance of *Penicillium* during the jointing stage and *Saitozyma* during the heading stage across both years. This suggests that T_3 enhances straw decomposition efficiency and nutrient turnover rates by continuously regulating the synergistic proliferation of *Penicillium* and *Saitozyma*.

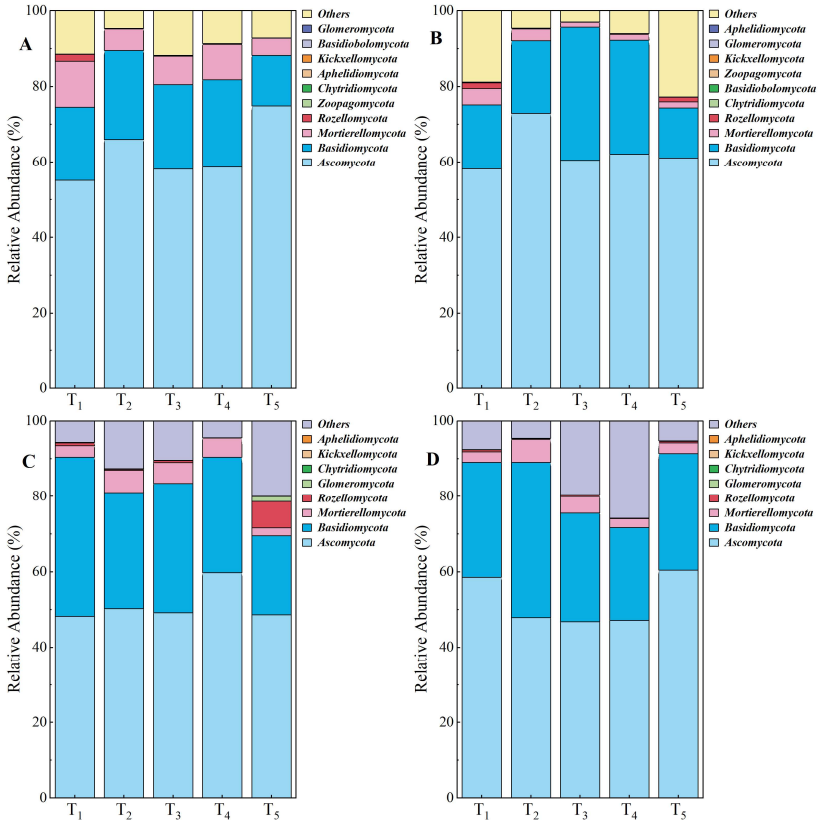


Figure 3. Changes in fungal species composition at the phylum level in soil. Note: (**A**): 2022 Jointing; (**B**): 2022 Heading; (**C**): 2023 Jointing; (**D**): 2023 Heading.

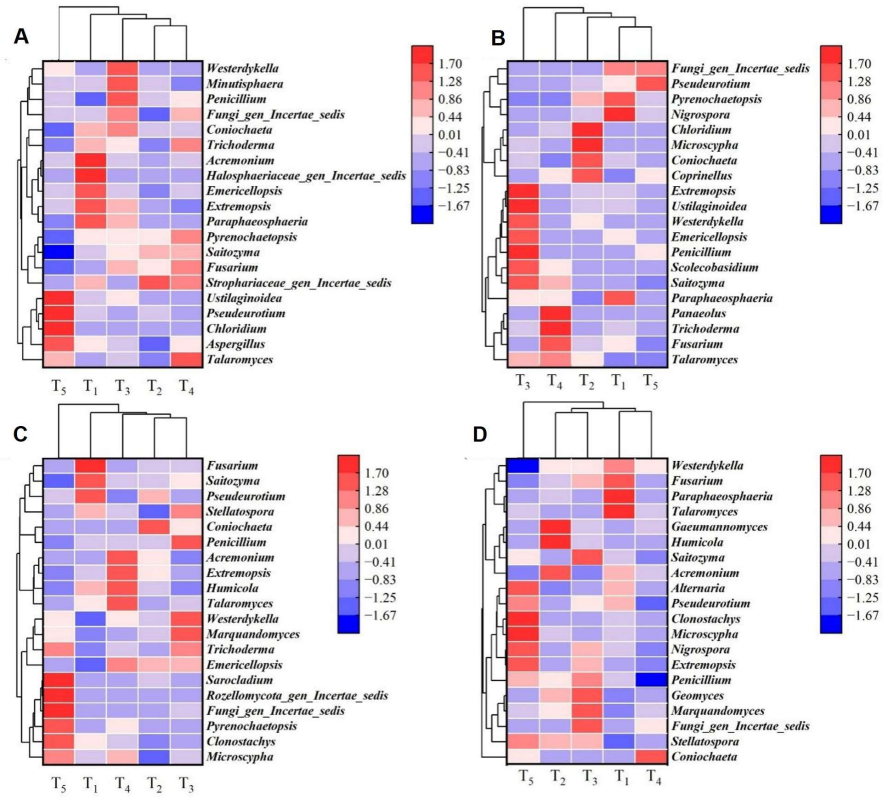


Figure 4. Relative abundance of fungi at the genus level in soil. Note: The meanings of notes (**A**–**D**) in the figures are the same as those in Figure 3. Color scale indicates relative abundance (%) ranging from 0 to maximum observed value per panel.

3.5. Correlations Between Soil Fungi and Rice/Soil Parameters

The two-year experimental results demonstrated significant correlations between soil fungal communities and rice dry matter accumulation, translocation, and soil nutrient content at different growth stages, exhibiting stage-specific dynamics and genus-specific variations (Figure 5). Overall, *Penicillium* and *Saitozyma* exhibited notable positive synergistic effects during the jointing and heading stages, respectively, whereas genera such as *Extremopsis* and *Microscypha* were negatively correlated with the measured parameters. During the jointing stages (Figure 5A,C), *Penicillium* showed highly significant positive correlations with leaf dry weight and soil total nitrogen content. *Penicillium* showed particularly strong associations with MTL, MCL, MTSS, ADM, GRP, total soil N, and ammonium N. These findings indicate a stable and positive regulatory role in yield formation during the early growth stages. Additionally, the genera *Fusarium* and *Saitozyma* exhibited positive correlations with most parameters, except MCSS and soil organic matter content, during the jointing stages of both years. Although the correlation strength for *Saitozyma* was slightly weaker, the trends remained consistent. In contrast, *Extremopsis* and *Microscypha* showed predominantly negative correlations with rice and soil indicators, suggesting their potential inhibitory effects.

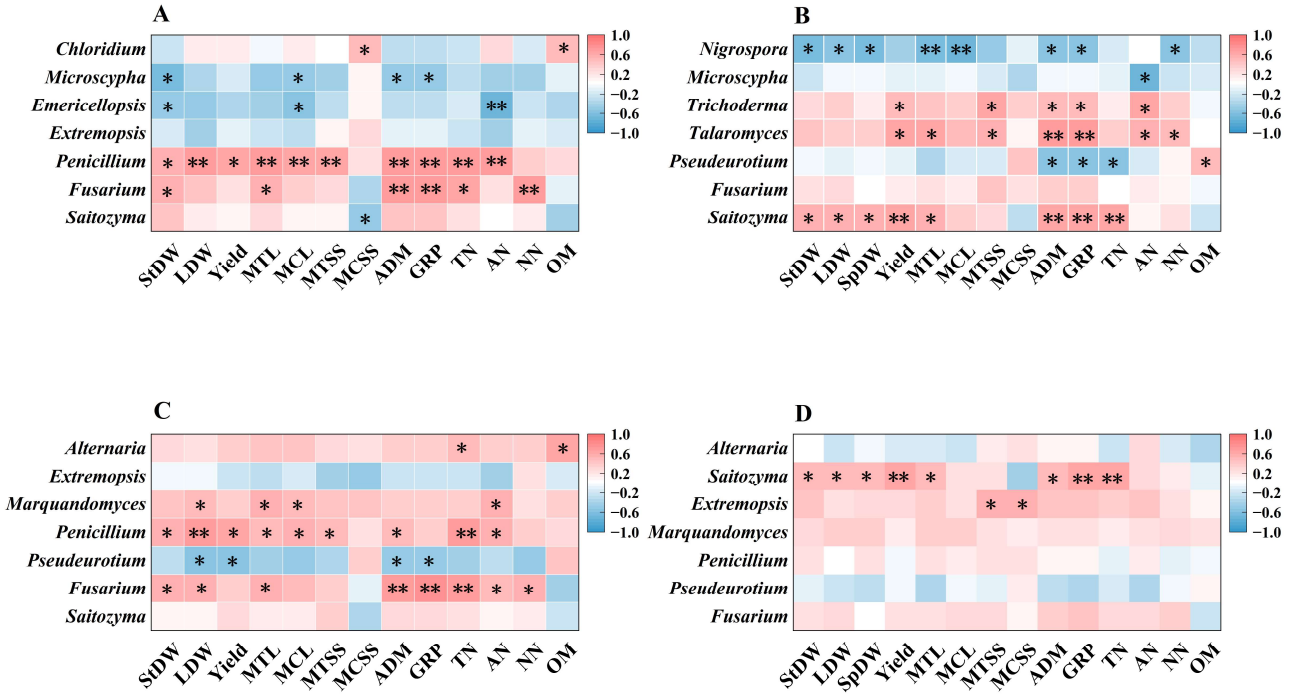


Figure 5. Correlation between soil fungi at genus level and rice indexes and soil indexes. Note: The meanings of notes (**A**–**D**) in the figures are the same as those in Figure 3. *, ** indicate significance at the 0.05 and 0.01 levels, respectively. StDW: Stem Dry Weight; LDW: Leaf Dry Weight; SpDW: Spike Dry Weight.

During the heading stages (Figure 5B,D), the fungal community structure underwent significant shifts. In 2022, *Trichoderma* and *Talaromyces* demonstrated significant positive correlations with rice yield and yield-related parameters, whereas *Nigrospora* and *Microscypha* showed predominantly negative correlations. Correlations involving the genus *Saitozyma* were strengthened during the heading stage, showing highly significant positive associations with yield and GRP. This reflects sustained synergistic effects during the later growth phases. During the 2023 heading stage, *Penicillium* and *Marquandomyces* maintained a positive correlation, though weaker than that observed at the jointing stage. *Saitozyma* showed consistent positive correlations with rice and soil parameters in both years that

intensified during the heading stage. *Saitozyma* exhibited significant positive correlations with rice yield, GRP, and total soil N content. These findings indicate that the abundance of the genera *Penicillium* at the jointing stage and *Saitozyma* at the heading stage is strongly correlated with both rice yield and soil physicochemical properties. These findings suggest that beneficial soil fungi during different rice growth stages can synergistically enhance crop yield and soil nutrient availability through coordinated interactions.

4. Discussion

4.1. Effects of Rice–Wheat Rotation, Wheat Straw Return, and Combined Application of Microbial Agents on Rice Growth and Soil Environment

Returning straw to the field is a pivotal strategy for enhancing farmland productivity and has positive effects on crop yield and nutrient utilization rates [24,25]. In this study, we showed that returning wheat straw to the field (T_2) significantly elevated rice yield by 3.80–10.06% compared with the treatment without straw return (T_1), which is consistent with the trends observed in previous studies. Wang et al. [26] demonstrated that, compared with the control, after straw return to the field rice yield increased by 3.50–7.65%, effective spikes by 7.77%, and thousand grain weight by 2.56%. Notably, straw carbonization increased the yield further by 5.64% [27], suggesting that straw treatment is critical in regulating fertilizer efficacy. The efficiency of straw decomposition directly affects the agricultural benefits. Inadequate decomposition rates may lead to a decline in crop yield. Recently, microbial agents have emerged as key regulatory tools [28,29]. In this study, wheat straw return to the field for the combined application of *Bacillus subtilis* and *Trichoderma harzianum* at a 1:1 ratio (T_3) led to an increase in rice yield; this synergistic effect might stem from multiple functions of the microbial agents. Rais et al. [30] demonstrated that inoculating the soil with *Bacillus* significantly minimized the secondary damage caused by rice blast. Han et al. [31] found that the inoculation of soil with *Bacillus subtilis* under non-saline and saline conditions promoted plant growth by directly or indirectly regulating chlorophyll content, leaf osmotic potential, cell membrane integrity, and ion accumulation. Furthermore, *Bacillus subtilis* volatile compounds have a lasting beneficial effect on *Arabidopsis* growth, improving the seed-setting rate. Chang et al. [32] demonstrated that the use of microbial preparations containing *Bacillus subtilis* and *Bacillus gelatinis* significantly increases plant height, tillering number, and secondary root number of wheat, promotes growth, and increases the number of spikes of wheat to a maximum of 10.2%, with an increase in yield of 7.74–24.23%. Lu et al. [33] demonstrated that bio-organic bacterial fertilizers enhance the lodging ability of rice. Reducing the amount of nitrogen fertilizer applied in paddy fields can increase the 1000-grain weight of rice and increase the yield by 2.03–9.88%. This further increase in yield may be attributed to microbial agents promoting straw decomposition, thereby accelerating the release of nutrients from the straw. Li et al. [34] demonstrated that the introduction of straw powder into a Petri dish culture of *Trichoderma* enhanced its ability to degrade wheat over time; compared to the control group, after four cultivation cycles at 28 °C, the degradation capacity peaked, with a straw powder weight loss rate of 19.75%. We found that the combined application of microbial agents promoted nutrient release by accelerating the decomposition of wheat straw, which was reflected in the changes in dry matter accumulation. Compared with the control treatment T_1, the dry matter weights of rice leaves in the T_2 treatment increased by 11.09–22.71%, throughout the growth period, and the accumulation of dry matter in the spikes increased by 1.59–11.38% at the maturity stage. On the basis of straw return, the additional application of microbial inoculants further enhanced the increase in dry matter accumulation, which may be partly attributed to improved nutrient availability. Among our treatments, T_3 exhibited the most significant increase in ammonium nitrogen during

the jointing and maturity stages, indicating its potential to enhance nitrogen supply and maintain its availability in the later stages of crop growth. The synergistic increase of total nitrogen and ammonium nitrogen under T_3 treatment provided a foundation for efficient nitrogen uptake and dry matter accumulation. In summary, the T_3 treatment primarily improved the supply efficiency of various nitrogen forms, particularly promoting the early accumulation of total nitrogen and ammonium nitrogen, ultimately leading to further increases in dry matter accumulation. Zhao et al. [35] and Li et al. [36] reported that straw return increased rice dry matter accumulation by 8.78–13.66%. The present study further demonstrates that co-application with microbial inoculants can amplify this effect.

Fungal communities play a key role in soil ecosystems. They not only participate in the decomposition of organic matter and nutrient cycling, but also affect the health of the soil and the growth of crops [37,38]. The change in environmental factors can significantly change the structure of soil fungal communities [39,40]. In the present study, after returning wheat straw to the field and combined application of microbial agents, the structure of the fungal community in the soil changed. The T_3 treatment showed the highest values for both the Shannon and Chao1 indices. This indicates that the richness and diversity of the soil fungal communities under this treatment were the most significant. Soil fungi participate in soil nutrient cycling by decomposing organic matter and affecting soil nutrient states [41]. Luo et al. [42] showed that the fungal genera *Nigrospora* and *Cladosporium* are significantly positively correlated with available soil phosphorus and potassium. Wang et al. [43] found that the application of microbial agents significantly increased the contents of total nitrogen, alkali-hydrolysable N, available P, and available K in the rhizosphere soil of continuously cropped flue-cured tobacco. Notably, certain fungi, such as those of the genus *Articulospora*, were positively correlated with the total N content and the increase in available P content. In this study, we found that T_3 treatment exhibited higher soil nutrient content than the other treatments. This may be related to the optimization of the soil fungal community structure under this treatment. For instance, the fungal genera *Penicillium* and *Saitozyma* were positively correlated with rice indicators and soil nutrient content. This indicates that these fungi may play important roles in promoting soil nutrient transformation and improving nutrient availability. Moreover, at different periods in T_3, the relative abundance of these fungal genera was significantly increased compared with the other treatments. This may explain the superior performance of the T_3 treatment, which further supplements and improves the results of Zhou et al. [44]. This indicates that the T_3 treatment can promote the decomposition of soil organic matter and the transformation of nutrients more effectively [45], aligning with the "microbial agents–enzyme activity–nutrient cycling" linkage mechanism proposed by Zhou et al. [44].

In conclusion, the return of wheat straw to the field and the combined application of microbial agents can increase rice yield by altering the structure of the soil fungal community, enhancing soil nutrient availability, and promoting root growth, as well as the accumulation of dry matter in the aboveground parts. Future research should further explore the effects of different ratios and application methods of microbial agents on soil fungal communities and their functions to optimize farmland management practices and promote sustainable agricultural development.

4.2. Improving Rice Yield and Soil Quality Through Wheat Straw Incorporation and Microbial Inoculants in a Rice–Wheat Rotation System

In recent years, with the continuous increase in crop yield, the amount of straw generated has increased substantially [46]. However, direct straw burning not only causes environmental pollution but also the loss of nutrients contained within the straw, preventing its reuse [47]. Therefore, the rational return of straw for recycling is an important agricultural practice. Previous studies have largely reported positive increases in crop

yields following straw return to the field [48,49]. However, a decrease in crop yield after straw return has also been reported [50], which may be attributed to the accumulation of straw and its low decomposition rate, both of which have negative impacts on the soil environment. Microorganisms, integral components of the soil ecosystem, when applied appropriately, can improve the soil microenvironment and enhance crop production [51,52]. Therefore, in the context of straw return, it is of great significance to explore how the rational application of microbial agents can promote straw decomposition, thereby improving crop yield and soil environment. Previous studies have widely reported the positive increase in crop yield following straw return [28,49]. This study also found that, compared to the no-return treatment, straw return increased rice yield. However, some studies have pointed out that slow straw decomposition, leading to accumulation in the field, may have negative effects on the soil environment, ultimately reducing crop yield [53,54]. Microorganisms, as core components of the soil ecosystem, can optimize the soil microenvironment and enhance crop yield when applied appropriately [51,52]. Microbial inoculants have been shown to effectively promote straw decomposition and enhance soil microbial community diversity [28], thereby improving the soil environment. In this study, the application of microbial agents and their effects on fungal genera revealed that the relative abundances of *Penicillium* and *Marquandomyces* during the jointing and mature stages were significantly higher in the T_3 treatment than in the other treatments. Moreover, the correlation analysis revealed that over the two years, during the jointing stage, both *Penicillium* and *Marquandomyces* were positively correlated with rice dry biomass, material transport, soil nutrients, and yield. Although the correlations weakened during the heading stage, they remained mostly positive. Notably, at the heading stage, the positive correlations of *Saitozyma* with rice dry biomass, material transport, total soil N, and yield were significantly stronger than those at the jointing stage.

Furthermore, rice yield is dependent on the accumulation of dry matter and its efficient transport to the spikes [55,56]. In this study, the T_3 treatment exhibited higher values of MTL, MTSS, ADM, and GRP from the heading to the maturity stage, indicating that the combined application of microbial agents based on wheat straw return to the field can not only enhance the photosynthetic product accumulation capacity of plants, but also improve the transport efficiency of nutrients from vegetative organs to reproductive organs. In particular, the material contribution rates of stem sheaths and leaves were significantly higher in the T_3 treatment than in the other treatments. This mechanism, which promotes the accumulation and transport efficiency of dry matter during the critical growth period of rice, represents an important pathway through which microbial agents contribute to rice yield increases and provides a strong foundation for the development of a high-yielding population structure.

In conclusion, the T_3 treatment demonstrated the most favorable outcomes in terms of rice yield. The combined application of microbial agents alongside wheat straw return to the field increases rice yield and improves the soil environment by modulating the soil microenvironment. This modulation increases the relative abundance of beneficial microbes, such as the bacterial genus *Anaeromyxobacter* and the fungal genera *Penicillium*, *Marquandomyces*, and *Saitozyma*. These microbes promote the efficient decomposition of wheat straw, improving soil nutrient levels and enhancing soil enzyme activities, leading to an increase in soil total nitrogen and nitrate nitrogen content. These effects promote rice biomass formation, ultimately contributing to higher yields.

5. Conclusions

This study found that wheat straw return can lay the foundation for increasing rice yield by altering the composition of soil fungal communities and enhancing the levels of

total nitrogen, nitrate nitrogen, ammonium nitrogen, and organic matter. On this basis, the combined application of *Bacillus subtilis* and *Trichoderma harzianum* at a dosage of 20 g m^{-2} further strengthens soil nitrogen supply, significantly promoting aboveground biomass accumulation and grain yield. The core mechanism underlying this effect lies in optimizing the soil fungal community structure, enriching the *Penicillium* genus during the jointing stage and the *Saitozyma* genus during the heading stage, enhancing straw decomposition and nitrogen transformation efficiency, and significantly increasing total nitrogen and ammonium nitrogen levels in the soil. At the same time, this treatment promotes efficient dry matter accumulation and transport, with significant increases in stem–leaf dry weight at heading and spike accumulation at maturity, as well as a synergistic increase in material transport efficiency and growth rate. This synergistic model, driven by microbial nutrient cycling and crop material allocation, provides both theoretical and practical support for sustainable high-yield rice cultivation.

6. Limitations of the Study

Uncertainty regarding the study period and long-term effects: This study is based solely on short-term experimental results from two years, without conducting long-term positioning trials. As such, it is unable to clarify the cumulative impacts of continuous straw return and microbial inoculant application on the evolution of soil fungal communities, nutrient pool balance, and crop yield stability over the long term.

Limited research on microbial community structure changes: The study offers a relatively limited examination of community dynamics and their ecological functions at different growth stages. Furthermore, there is insufficient exploration of the specific mechanisms underlying soil nitrogen transformation processes. Future studies should delve deeper into how the combined application of microbial inoculants influences the activity of specific microbial communities, thereby regulating key soil processes such as nitrogen mineralization, nitrogen fixation, and nitrification.

Experimental conditions and applicability: The experiment was conducted under the ecological conditions of Sichuan Province with a wheat straw return rate of 4710 kg hm^{-2} (10% moisture content). The applicability of the best microbial inoculant combination (1:1 *Bacillus subtilis* and *Trichoderma harzianum*, 20 gm^{-2} application rate) needs further validation under different ecological regions and soil conditions. The study focused solely on this specific ratio, dosage, and application method, without exploring optimization of other parameters. The results are also constrained by specific soil and climate conditions, and a lack of cross-regional validation limits their generalizability.

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Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

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