Article

Low-Temperature Fermented Straw Compost Regulates Rice Growth and Yield by Affecting Soil Physicochemical Properties and the Expression of Important Signaling Pathway Genes

Tongtong Liu [1,†]**, Ziguang Liu** [2,†]**, Ziyi Zhao** [1,†]**, Kai Xu** [1]**, Heshu Chen** [2]**, Yanzhong Feng** [2]**, Wentao Wang** [2]**, Nan Zhang** [1,3]**, Di Liu** [2]**, Xinmiao He** [2,*] **and Juan Wu** [1,*]

1. Key Laboratory of the Ministry of Education for Ecological Restoration of Saline Vegetation, College of Life Sciences, Northeast Forestry University, Harbin 150040, China
2. Key Laboratory of Combining Farming and Animal Husbandry, Institute of Animal Husbandry of Heilongjiang Academy of Agricultural Sciences, Ministry of Agriculture and Rural Affairs, Harbin 150086, China
3. Graduate School of Science, Nagoya City University, Nagoya 467-8501, Japan
* Correspondence: haashxm@163.com (X.H.); wuj1970@163.com (J.W.)
† These authors contributed equally to this work.

Abstract: Soil physicochemical properties affect crop growth and yield. The addition of fertilizers can improve the soil quality during crop cultivation, leading to increased agricultural production. Organic fertilizers may be produced by composting straw that would otherwise be discarded as agricultural waste, with potential implications for sustainable agricultural development. However, the mechanism underlying the effects of straw compost on crop growth is unknown. In this study, a microbial agent suitable for straw decomposition in cold regions was used for a large-scale biological fermentation. Organic compost was obtained after the decomposition of straw. The straw compost was mixed with soil in different proportions and then used to cultivate Songjing 2 rice plants. The addition of straw compost significantly increased the growth and yield of the rice plants and enhanced various physiological indices. Moreover, the straw compost treatment significantly improved soil physicochemical properties (e.g., pH, enzyme activity, nutrient composition, and microbial diversity) and optimized the soil conditions for crop growth. In addition, the application of straw compost influenced the expression of genes in rice metabolic pathways as well as pathways mediating secondary metabolite synthesis and plant hormone signal transduction. The study data reflect the potential applicability of low-temperature straw fermentation technology for maximizing crop production.

Keywords: straw compost; rice; soil physicochemical properties; soil microbial diversity; transcriptome sequencing

1. Introduction

Soil is a valuable agricultural resource comprising many components important for the cultivation of agriculturally important crops [1]. During crop cultivation, soil nutrients are lost with each harvest. Accordingly, if agricultural soils are not managed appropriately, the associated decrease in soil fertility will adversely affect crop growth, resulting in crop yield losses, while also threatening sustainable agricultural development [2]. Therefore, improving soil quality to increase crop yields is crucial for maintaining food security and sustainable ecosystem development. Traditional methods for improving soil quality include physical and chemical treatments [3]. The chemical-based methods typically involve the application of inorganic fertilizers to increase the crop yield, but they may have detrimental effects on future agricultural production. Moreover, the excessive application of chemical fertilizers can destabilize the soil environment and damage ecological systems.

Straw, which refers to the cereal crop material remaining after the seeds are harvested, is the main by-product of agricultural production [4]. Northeastern China produces a large amount of straw every year because it serves as a major cereal-producing region [5]. The improper treatment of straw can damage the environment. For example, the air pollution caused by burning straw hinders ecological development. Furthermore, the particulate matter generated by incineration may be harmful to human health [6]. Straw contains organic carbon and a substantial abundance of nutrients essential for crop growth, including nitrogen, phosphorus, and potassium. The proper treatment of straw can turn it into a renewable biomass resource critical for preventing soil desertification and improving agricultural soils [7]. Straw composting can optimize the utility of straw resources. More specifically, it involves the manual and stable decomposition of straw to produce an organic fertilizer. Straw can be directly returned to the field or incinerated and composted and returned to the field [8]. Among these options, composting is likely the most common. Microorganisms are important for composting straw [9]. Microbial straw composting is a process that exploits the biochemical activities of microorganisms isolated from nature to convert the organic matter in plant waste to stable humus. For example, in bio-straw reactors, thermophilic microbial strains are used for the rapid and efficient retting of straw to generate a high-quality organic fertilizer [10]. In addition, earlier research on straw disintegration examined the use of plant endophytic bacteria as the core microbes and maximized the efficient recycling of the nutrients and organic matter in straw by modulating crop microecology and soil microorganisms. Yin et al. identified *Clonostachys rosea* strain YZC3 and determined that it can efficiently degrade straw lignin. Additionally, YZC3 can significantly promote straw composting, increase the humic acid content of compost products, and restrict the loss of nitrogen during composting. Hence, it is a potentially useful bacterial strain for composting [11]. Zhang et al. analyzed the compost of garden waste and isolated a *Geobacillus stearothermophilus* strain able to effectively degrade lignin at high temperatures. The laccase and lignin peroxidase activities were as high as 12.26 and 42.41 U/mL, respectively, and the waste lignin degradation rate reached 20.1%. Thus, this bacterium may be applicable for the industrial production of lignin-degrading bacterial agents [12]. Wan et al. recently identified mesophilic (MSDA1) and thermophilic (HDGA2) microbial strains with significant laccase, lignin peroxidase, and manganese peroxidase activities; both strains can efficiently degrade lignin, making them useful for the directed humification during garden waste composting [13]. The biodegradation of straw is a common practice because of its advantages (e.g., high degradation rate, environmentally friendly process, and low cost). Furthermore, it has become a major topic of interest among researchers focused on agricultural waste development and utilization [14].

The organic straw compost derived from biodegradation is a pure plant-based fertilizer that can significantly alter soil physical properties, nutrient dynamics, and vegetation establishment, thereby improving soil productivity and increasing crop yields [15]. Li et al. reported that corn straw compost can be used to decrease the pH and conductivity in saline-alkali soil, increase soil nutrient contents (e.g., available nitrogen, phosphorus, and soluble organic carbon), increase sucrase and urease activities, and improve the microbial community, thereby increasing the biomass- and yield-related characteristics of rice grown in saline-alkali soil (e.g., plant height, root length, and 1000-grain weight) [16]. The composting of rice straw using *Trichoderma* species can promote rice germination and increase seedling vigor and the total chlorophyll content, while also enhancing the production of various defense-related enzymes, such as catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD), ultimately increasing the stress resistance of rice [17]. The integration of wheat straw biochar compost and biogas slurry can positively affect the cation exchange capacity of soil, while increasing soil carbon, phosphorus, and potassium contents, soil microbial diversity, and urease and β-glucosidase activities. Moreover, applying biochar along with compost and biogas slurry can increase the maize plant height, chlorophyll content, water use efficiency, and 1000-grain weight [18]. Pang et al. showed that including a mixture of microbial agents during the application of biochar can improve the stress

resistance of rice plants growing in saline-alkali soil [19]. Therefore, the application of organic straw compost in agricultural fields can significantly improve the soil matrix state, increase crop yields, and promote sustainable agricultural development.

Although organic straw composting positively affects agricultural production, there has been relatively little research on the mechanism underlying its effects on crops. In this study, the effects of straw compost on the growth and development of Songjing 2 rice as well as soil physicochemical properties were investigated using straw compost treated with microbes mediating low-temperature fermentation [20]. The use of straw compost significantly increased Songjing 2 rice growth and yield, while also improving various plant physiological indices. Additionally, the application of straw compost improved soil quality, increased the activities of important soil enzymes, and improved the soil microbial community composition. A transcriptome analysis indicated that the addition of straw compost affected the expression of genes related to rice metabolic pathways, secondary metabolite biosynthesis, plant–pathogen interactions, and plant hormone signal transduction. In conclusion, the study findings provide the theoretical basis for the industrialization of this low-temperature straw fermentation technology and the extensive application of straw compost to improve agricultural production.

2. Materials and Methods

2.1. Experimental Materials in This Study

The straw compost and soil used in this research were taken from the Animal Husbandry Innovation Base of the Animal Husbandry Institute of Heilongjiang Academy of Agricultural Sciences (45°40′ N, 126°33′ E). A microbial agent suitable for straw decomposition in cold areas was used to ferment and decompose waste crop straw added with Longmin black pig feces for about 120 days. This microbial agent was composed of cold resistant fungi with cellulose degrading properties, including *Penicillium chrysogenum*, *Trichoderma citrinoviride*, and *Neurospora sitopila* [20]. The waste crop straw was composted into a rotten state, and occasionally strips or small fragments of incompletely decomposed straw were found. Unnecessary stones and plastic bags were removed, and the straw compost for subsequent experiments was obtained (Supplementary Figure S1). The cultured soil layer of 0–20 cm was collected, which was air-dried, crushed, and mixed before being used in pot experiments. The soil type was loam soil with loose texture and containing granular material. Treatment groups consisted of soil mixed with straw compost at ratios of 10%, 30%, and 50% (*v/v*), while the control group comprised regular soil alone (Supplementary Table S1) [16]. Other fertilizers were not used during the cultivation.

In this study, Songjing 2 rice, a japonica rice cultivable in north China, was selected, and was provided by the Tillage and Cultivation Institute of Heilongjiang Academy of Agricultural Sciences. In the experiment, 10 Songjing 2 rice seeds from the same batch were soaked in 3% NaClO (Tianjin Beilian Fine Chemicals Development Co., Ltd., Tianjin, China) for 15 min and 75% alcohol (Dezhou Chengze Disinfection Technology Co., Ltd., Dezhou, China) for 3 min, and washed with sterile water for 5 times. [21]. The sterilized seeds were planted in plastic buckets (upper diameter × bottom diameter × height = 25 cm × 17 cm × 18 cm), and each bucket was filled with 3 kg soil. The rice was cultivated under natural conditions from early May to early October. Rice irrigation was carried out throughout the entire growth period by flooding 1–4 cm. Various biomass and yield parameters including plant height, root length, seed size, and 100–grain weight were subjected to analysis. The experiments were conducted at a campus of Northeast Forestry University in Harbin, Heilongjiang Province, China (47°16′ N, 123°55′ E). Each experiment was set up with three treatment groups and each experiment was repeated three times (2021–2023).

2.2. Measurement of Rice Physiological Indices

According to the instructions provided by the kit (Nanjing Institute of Construction Bioengineering, Nanjing, China), about 0.1 g of fresh rice leaf samples at the booting stage under different growth conditions were used for measurement of physiological indices. The

content of soluble protein, activities of stress response-related catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), and superoxide dismutase (SOD) were evaluated, and hydrogen peroxide (H_2O_2) content was detected. The effect of straw compost on the plant chlorophyll content of the leaf was evaluated as per Porra [22].

2.3. DAB (3,3′-Diaminobenzidine) and NBT (Nitrotetrazolium Blue Chloride) Staining of Rice Leaves

Rice leaves of the tillering stage with good growth and suitable size were added with DAB staining solution [containing 0.1 mg DAB (Yeasen Biotechnology Co., Ltd., Shanghai, China), 1 mL 0.05 M Tris–HAC (Tris (hydroxymethyl) aminomethane), pH5.0] and NBT staining solution [containing 0.5 mg NBT (Shanghai Biyuntian Biotechnology Co., Ltd., Shanghai, China), 1 mL 25 mM HEPES (N-2-hydroxyethyl piperazine-N′-2-ethanesulfonic acid), pH7.6], respectively. After the reaction in the dark for about 24 h, the staining solutions were removed, bleaching solution (80% ethanol) was used for boiling decolorization, and photos were taken [23].

2.4. Measurement of Grain Shape and Hundred-Grain Weight of Songjing 2 Rice

Twenty rice grains from the control and treatment groups were randomly selected to compare the length and width of grains using the ImageJ software (version 1.51). Meanwhile, 100 rice grains from the control and treatment groups were randomly selected and measured for hundred-grain weight using an electronic scale (AS220.R2, RADWAG Wagi Elektroniczne, Radom, Poland). All experiments were performed with three biological replicates.

2.5. Determination of Soil Physiochemical Properties

Soil samples from rice rhizosphere at the tillering stage were collected for determination of physicochemical properties. The soil sample was fully mixed with water at a ratio of 1: 2.5 (*w/v*) and centrifuged at room temperature for 15 min to obtain a soil–water suspension [16]. The acidity of saturated soil extracts was determined in soil–water suspension using a pH meter.

The content of total nitrogen (TN) in the soil was determined by the Kjeldahl method. The content of total phosphorus (TP) in the soil was determined by molybdenum blue colorimetry, sulfuric acid digestion, and perchloric acid digestion after humidification. All the results were calculated on a dry basis [24]. The content of total organic carbon (TOC) in the soil was determined by the potassium dichromate volumetric method [25].

The activities of sucrase and urease in the soil were determined according to previously reported methods. The activity of sucrase in the soil was determined by 3, 5-dinitrosalicylic acid colorimetry at 508 nm. By using disodium phenyl phosphate as the substrate and measuring at A660 with the spectrophotometer, disodium phenyl phosphate colorimetry was used to detect the phosphatase activity in the soil [26]. Cellulase activity in the soil was detected by 3, 5-dinitrosalicylic acid colorimetry [27].

2.6. Determination of Soil Microbial Diversity

The bacteria, fungi, and actinomyces were determined by the plate coating counting method. Soil samples collected from rice rhizosphere at the tillering stage were screened and air-dried in time. Potato sucrose medium was used for the determination of fungi, beef extract peptone medium was used for the determination of bacteria, and Gao's medium No. 1 was used for the determination of actinomyces [28]. The prepared soil diluent was coated and cultured on a plate. Bacteria and actinomycetes were cultured inversely at 28 °C for 7–10 days, and fungi were cultured at 25 °C for 3–5 days. Microscopic examination and counting were performed, and the number of soil microorganisms was calculated according to the number of soil microorganisms (CFU/g) = (average number of colonies × dilution ratio) ÷ soil drying quality [28].

2.7. Rice Transcriptome Sequencing and Data Analysis

The rice leaves at the booting stage were harvested for transcriptome sequencing. The cDNA library for transcriptomic sequencing was constructed and sequenced by Metware (Wuhan, China). Single-stranded circle DNA molecules were replicated via rolling cycle amplification, and a DNA nanoball (DNB) which contains multiple copies of DNA was generated. Sufficient quality DNBs were then loaded into patterned nanoarrays using the high-intensity DNA nanochip technique and sequenced through combinatorial Probe-Anchor Synthesis (cPAS). The original data were filtered using fastp [29], and high-quality reads were then mapped to the genome using HISAT [30]. DESeq2 [31] was used to analyze the differential expression between the two groups, and genes satisfying the criteria of a $|\text{Log2FoldChange}| \geq 1$ and FDR (False Discovery Rate) < 0.05 were identified as differentially expressed genes (DEGs). KEGG, GO, and KOG annotations were performed, and enrichment analysis was performed using cluster Profiler.

2.8. qRT-PCR Analysis

Total RNAs of the rice leaves at the booting stage were extracted with trizol Reagent (Invitrogen, Waltham, MA, USA). cDNA was prepared from 5 μg total RNA at various conditions using the Prime Script RT Reagent Kit with gDNA Eraser (Takara Bio, Kusatsu, Japan). Next, 50 ng cDNA and 40 nM primers (Supplementary Table S2) were used for each RT-qPCR reaction with 2× Brilliant III SYBR Green QPCR Master Mix (Agilent, Santa Clara, CA, USA) and a Mx3000P Real-Time Thermal Cycling System (Agilent, Santa Clara, CA, USA). Each expression level was normalized with the *OseEF-1a* gene [32].

2.9. Statistical Analysis

The statistical analyses for all tables were performed using R software (version 4.0.1) and two-tailed paired *t*-tests; $p < 0.05$ was considered statistically significant.

The data obtained in qRT-PCR analysis were the mean of three biological replicates. The data were analyzed using one-way analysis of variance by SPSS, and statistically significant differences were calculated based on Student's *t*-test, with $p < 0.05$ (*) and $p < 0.01$ (**) as thresholds for significance.

3. Results

3.1. Straw Compost Affected the Rice Biomass and Yield

Previous studies revealed the importance of straw compost for improving crop growth and increasing yields [16]. Therefore, we investigated the effects of low-temperature fermented straw compost on Songjing 2 rice. Under various planting conditions, the control plants and the plants treated with 10%, 30%, and 50% straw compost were similar in height for the first 5 days of growth. Additionally, there was no significant difference in rice morphological characteristics. However, after 3 months of growth, the plants treated with straw compost were significantly taller than the control plants. Specifically, the plant height increased significantly as the amount of straw compost applied increased (Figure 1A and Figure S2). The roots of the plants treated with 30% and 50% straw compost were significantly longer than the roots of the control plants (Figure 1B). The rice grains of the plants treated with 30% straw compost were significantly longer and wider than the rice grains of the control plants (Figure 1D). Accordingly, the 100-grain weight was significantly greater for the rice plants treated with 30% straw compost than for the control rice plants (Figure 1E).
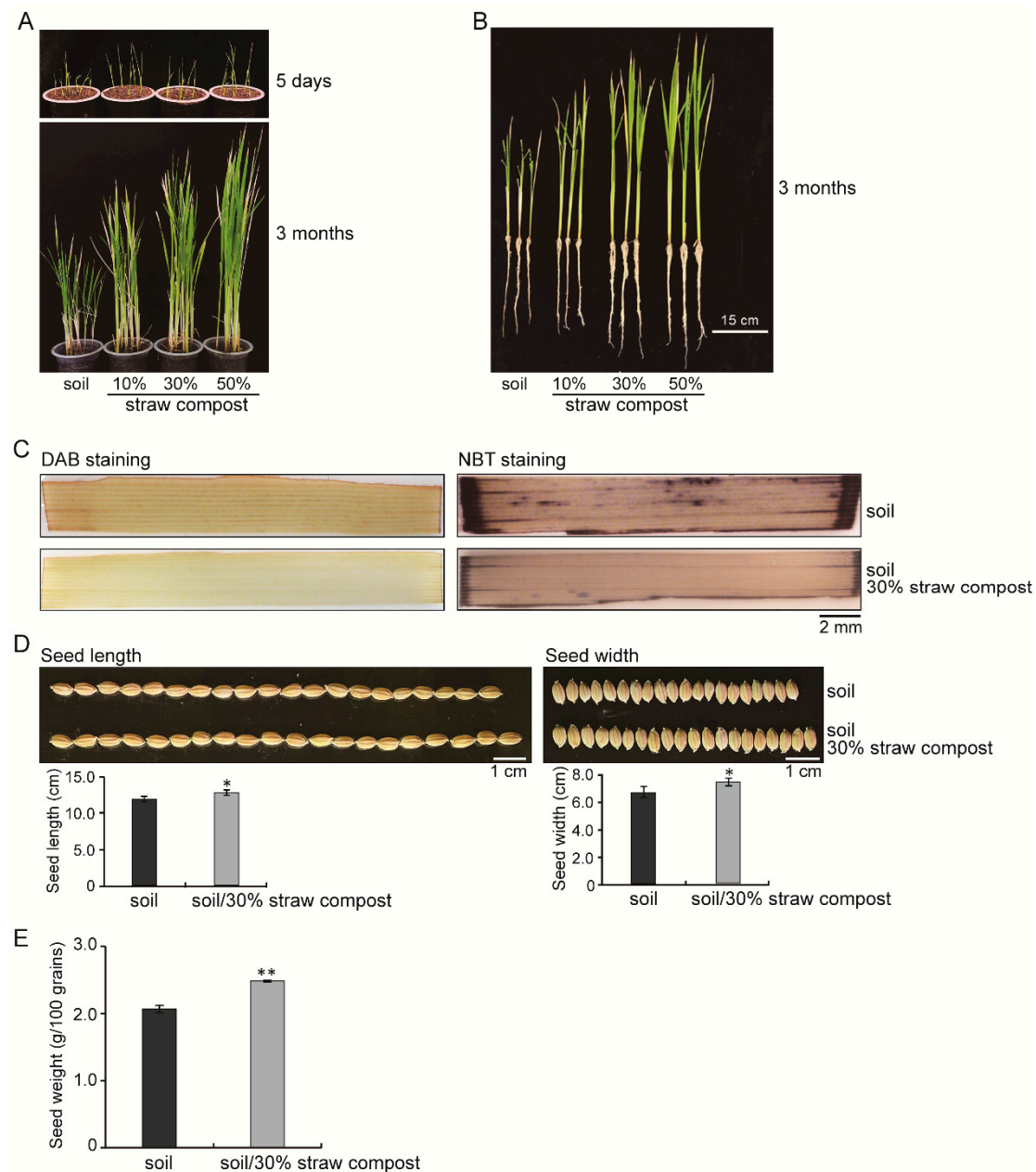
Figure 1. The effects of straw compost on biomass, yield, and physiological indices of Songjing 2 rice. (**A**) The seedlings of Songjing 2 rice grown in normal soil for 7 days were transferred to normal soil and normal soil with 10%, 30%, and 50% straw compost for growing for 5 days and 3 months, the growth status of the Songjing 2 rice was observed, and photos were taken. (**B**) The morphology of Songjing 2 rice grown for 3 months on normal soil and normal soil with 10%, 30%, and 50% straw compost. (**C**) The effect of straw compost on the H_2O_2 and superoxide anion content in the Songjing 2 rice leaves at booting stage. Rice leaves of tillering stage with good growth were added with DAB and NBT staining solution in the dark for about 24 h, then 80% ethanol was used for boiling decolorization and photos were taken. (**D**) Comparative analysis of rice grain sizes from normal soil and soil with straw compost. Each data value represented the mean $\pm$ SE of three replicates, with each using 20 rice grains. (**E**) Comparative analysis of grains yield from normal soil and soil with straw compost. Each data value represented the mean $\pm$ SE of three replicates, with each using 100 grains weight. Asterisks indicated statistically significant differences compared with the normal soil: * $p < 0.05$ and ** $p < 0.01$ as determined by the Student's *t*-test.

3.2. Effects of Straw Compost on Various Rice Physiological Indices

Because earlier research indicated that the application of straw compost affects various physicochemical properties of crops [33], we analyzed the effects of straw compost fermented under cold conditions on Songjing 2 rice. Soluble proteins are important osmoregulatory substances and nutrients that can protect cell components and biofilms [34]. The addition of 30% straw compost slightly increased the soluble protein content of the rice leaves at the tillering stage (Table 1). POD, which is an important scavenger of reactive oxygen species, is a key contributor to plant stress resistance. The POD activity was more than two times higher in the plants treated with 30% straw compost than in the control plants (Table 1). Similarly, the activity of CAT, which is a key enzyme for the decomposition of hydrogen peroxide (H_2O_2), was more than two times higher in the plants treated with 30% straw compost than in the control plants (Table 1). Both APX and SOD are important antioxidant enzymes that protect plants by modulating reactive oxygen species metabolism and eliminating free radicals to maintain cellular homeostasis. The addition of 30% straw compost increased the APX and SOD activities, while also decreasing the leaf H_2O_2 content to one-third of the level in the control plants (Table 1). Thus, the straw compost treatment significantly increased the activities of stress response-related enzymes and enhanced the stress resistance of rice.

Table 1. Effects of straw compost on various physiological indices of Songjing 2 rice.

Sample	Soluble Protein Content (μg/mL)	POD Activity (U/mg)	CAT Activity (U/mg)	APX Activity (U/mg)	SOD Activity (U/mg)	H_2O_2 Content (μmol/mL)
Soil	44.074 ± 2.631 [a]	23.314 ± 1.066 [b]	0.054 ± 0.004 [b]	0.005 ± 0 [b]	1.209 ± 0.007 [b]	0.213 ± 0 [a]
Soil/30%-straw compost	47.909 ± 1.047 [a]	49.239 ± 0.482 [a]	0.113 ± 0.014 [a]	0.006 ± 0 [a]	1.444 ± 0.058 [a]	0.075 ± 0.005 [b]

Means sharing similar letter (s) in a column do not differ significantly at $p = 0.05$; Data are average of three replicates ± SE.

We used DAB and NBT staining methods to investigate the effects of straw compost on the H_2O_2 and superoxide anion contents in rice leaves. The DAB staining results indicated the brown spots were significantly darker on the leaves of the control plants than on the leaves of the plants treated with straw compost (Figure 1C). Similarly, among the NBT-stained samples, the blue spots were darker on the leaves of the control plants than on the leaves of the plants treated with straw compost (Figure 1C).

The leaf chlorophyll content can reflect the photosynthetic capacity of the plant. The leaf chlorophyll *a* and *b* contents at the tillering stage were, respectively, 1.25 and 1.12 times higher in the plants treated with 30% straw compost than in the control plants. The leaf carotenoid content was nearly 1.3 times higher in the plants treated with 30% straw compost than in the control plants (Table 2).

Table 2. Effects of straw compost on Songjing 2 rice leaf quality.

Sample	Chlorophyll a (μg/mL)	Chlorophyll b (μg/mL)	Carotenoid (μg/mL)
Soil	8.81 ± 0.24 [b]	3.54 ± 0.30 [a]	2.96 ± 0.06 [b]
Soil/30%-straw compost	11.04 ± 0.05 [a]	3.98 ± 0.09 [a]	3.81 ± 0.22 [a]

Means sharing similar letter (s) in a column do not differ significantly at $p = 0.05$; Data are average of three replicates ± SE.

3.3. The Application of Straw Compost Improved the Soil Physicochemical Properties

Soil quality directly affects crop yield. Therefore, we collected rice rhizosphere soil samples at the booting stage of plants grown under various conditions for an analysis of physicochemical properties. The pH was lower for the control soil (4.87; slightly acidic) than for the soil supplemented with 30% straw compost (6.35). The organic matter and total phosphorus contents were significantly higher in the soil containing straw compost

than in the control soil. The total nitrogen content was slightly higher in the soil containing straw compost than in the control soil (Table 3).

Table 3. Soil physicochemical properties under straw compost.

Sample	Soil pH	Organic Matter (g/kg)	Total Nitrogen (N) %	Total Phosphorus (P) (g/kg)
Soil	4.87 ± 0.12 [b]	206 ± 6.08 [b]	0.73 ± 0.04 [a]	0.63 ± 0.02 [b]
Soil/30%-straw compost	6.35 ± 0.02 [a]	252 ± 4.58 [a]	0.87 ± 0.10 [a]	1.11 ± 0.03 [a]

Means sharing similar letter (s) in a column do not differ significantly at $p = 0.05$; Data are average of three replicates $\pm$ SE.

Various soil enzymes are important for the material cycling and energy conversion that influence crop growth. Therefore, we examined the sucrase, cellulase, urease, and phosphatase activities, which are closely related to many soil factors. In the soil supplemented with 30% straw compost, the sucrase activity was nearly double that in the control soil. Moreover, the cellulase and phosphatase activities were, respectively, nearly 5 and 1.3 times higher in the soil containing 30% straw compost than in the control soil. In contrast, the urease activity was only slightly higher in the soil supplemented with 30% straw compost than in the control soil (Table 4). Hence, the addition of straw compost improved the soil physicochemical properties.

Table 4. The effect of straw compost on enzyme activity of soil.

Sample	Sucrase Gmg/(g·24 h)	Urease mg/(g·24 h)	Cellulase µg/(mL·min)	Phosphatase mg/(g·24 h)
Soil	8.48 ± 0.11 [b]	1.15 ± 0.03 [b]	0.46 ± 0.03 [b]	25.59 ± 0.12 [b]
Soil/30%-straw compost	15.17 ± 0.09 [a]	1.45 ± 0.05 [a]	2.28 ± 0.03 [a]	33.00 ± 1.73 [a]

Means sharing similar letter (s) in a column do not differ significantly at $p = 0.05$; Data are average of three replicates $\pm$ SE.

3.4. The Addition of Straw Compost Increased the Soil Microbial Abundance

Soil microorganisms are an indispensable part of the soil ecosystem. They play an important role in the degradation of organic matter and the improvement of the soil structure, with key effects that maintain the soil quality and promote plant growth. Thus, the microbial abundance in the rice rhizosphere soil at the tillering stage was determined. The bacterial abundance in the soil supplemented with 30% straw compost (2.30×10^7 CFU/g) was nearly double that in the control soil. Specifically, the soil containing 30% straw compost had three times more actinomycetes (4.27×10^5) than the control soil. However, the number of fungi was not significantly affected by the addition of 30% straw compost (Table 5). Overall, the addition of straw compost increased the soil microbial richness.

Table 5. The effect of straw compost on soil microbial abundance.

Sample	Bacteria (CFU/g)	Fungus (CFU/g)	Actinomycetes (CFU/g)
Soil	$1.53 \times 10^7 \pm 3.46 \times 10^5$ [b]	$7.07 \times 10^4 \pm 9.53 \times 10^2$ [a]	$1.19 \times 10^5 \pm 9.64 \times 10^3$ [b]
Soil/30%-straw compost	$2.30 \times 10^7 \pm 2.65 \times 10^5$ [a]	$7.08 \times 10^4 \pm 4.58 \times 10^2$ [a]	$4.27 \times 10^5 \pm 1.35 \times 10^4$ [a]

Means sharing similar letter (s) in a column do not differ significantly at $p = 0.05$; Data are average of three replicates $\pm$ SE.

3.5. Changes in the Rice Leaf Transcriptome in Response to the Straw Compost Treatment

To clarify the mechanism underlying the positive effects of the straw compost on rice growth, a transcriptome sequencing (RNA$-$seq) analysis was performed using rice

leaves collected at the booting stage. High-quality reads were obtained, with an average GC content of 52.35% (Supplementary Figure S3), suggesting the obtained data were appropriate for the subsequent analysis. A total of 2473 genes were differentially expressed between the control Songjing 2 rice plants and the plants treated with straw compost (Supplementary Table S3). Among these differentially expressed genes (DEGs), 982 and 1491 had up-regulated and down-regulated expression levels, respectively, in the straw compost-treated plants (Figure 2A). The analysis of the 15 most up-regulated and down-regulated genes suggested many processes in rice were affected by the straw compost treatment (Figure 2B).
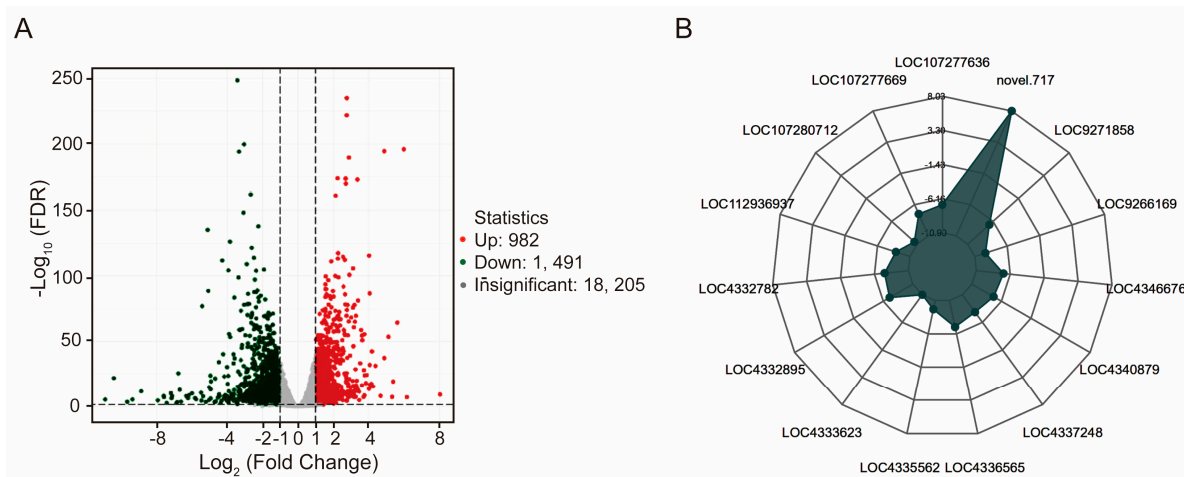

Figure 2. The DEGs analysis of Songjing 2 rice using RNA−seq data under straw compost. (**A**) Volcano plot of DEGs of Songjing 2 rice under straw compost. The horizontal axis represented the changes in gene expression multiples, the vertical axis represented the significance level of DEGs. The red dots represented up−regulated DEGs, the green dots represented down−regulated DEGs, and the gray dots represented non differentially expressed genes. The number of up−regulated, down−regulated, and non−differentially expressed genes is indicated on the right side. (**B**) The radar map of the 15 up-regulated and down-regulated genes with the highest multiple differences. Each point in the map represented a gene, and the position of the point corresponded to the size of the log2fold change value of the gene.

A GO enrichment analysis was completed to functionally annotate the DEGs identified following the straw compost treatment. The significantly enriched GO terms among the DEGs included the following: iron ion binding, monooxygenase activity, UDP-glycosyltransferase activity, hexosyltransferase activity, isoprenoid metabolic process, isoprenoid biosynthetic process, monocarboxylic acid biosynthetic process, response to oxidative stress, secondary metabolite biosynthetic process, terpenoid metabolic process, response to hypoxia, cellular response to decreased oxygen levels, cellular response to oxygen levels, response to decreased oxygen levels, and response to oxygen levels (Figures 3 and S4). Accordingly, the DEGs were associated with enzyme activities, metabolism and biosynthesis, and stress responses.
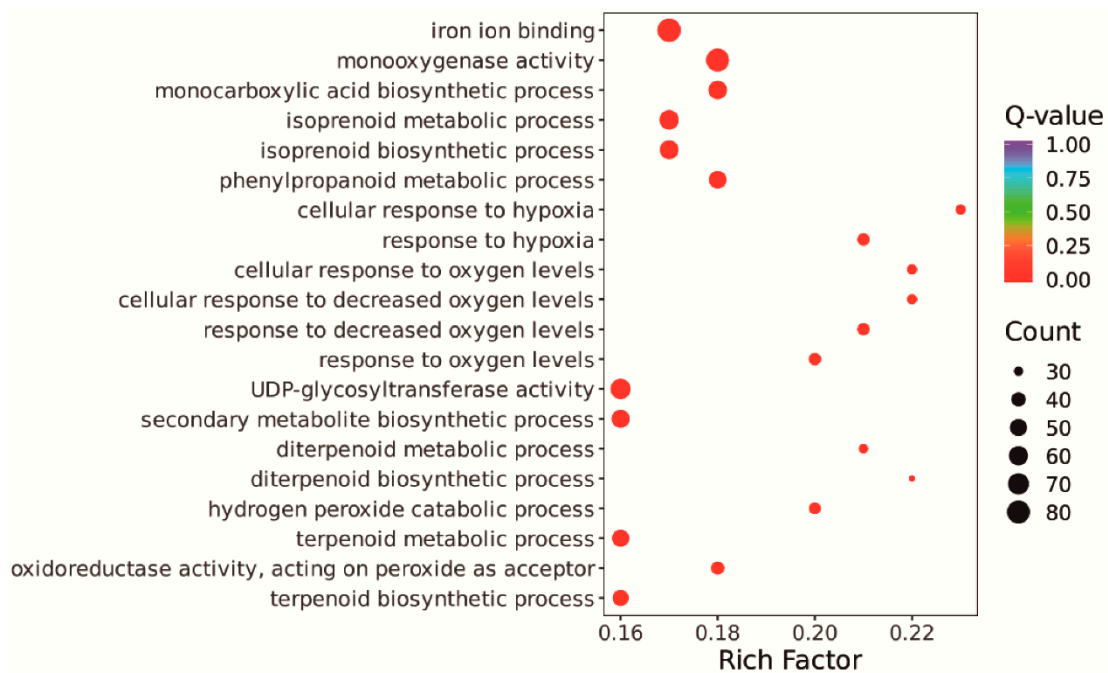
Figure 3. The GO enrichment analysis of DEGs of Songjing 2 rice under straw compost. The vertical axis represented the GO entry, and the horizontal axis represented the rich factor. The larger the rich factor, the greater the degree of enrichment. The larger the dot, the more DEGs there are in GO entries. The redder the color of the dot, the more significant the enrichment.

To further elucidate the effects of the straw compost treatment on rice gene expression, the DEGs underwent a KEGG (Kyoto Encyclopedia of Genes and Genomes) analysis. The results indicated that 356 DEGs were involved in metabolic pathways (ko01100), 274 DEGs were involved in the biosynthesis of secondary metabolites (ko01110), 53 DEGs were involved in phenylpropanoid biosynthesis (ko00940), 162 DEGs were involved in plant–pathogen interactions (ko04626), 104 DEGs were involved in plant hormone signal transduction (ko04075), and 85 DEGs were involved in MAPK signaling (ko04016) (Figure 4 and Figure S5). Thus, many of the DEGs in the rice plants treated with straw compost were associated with metabolic activities and the biosynthesis of secondary metabolites.

The DEGs were also mapped using the KOG database (containing eukaryotic orthologous groups). A total of 1215 DEGs were classified into 23 KOG categories. The largest category was general function prediction only (187, 15.39%), followed by signal transduction mechanisms (141, 11.6%), secondary metabolites biosynthesis, transport and catabolism (127, 10.45%), and posttranslational modification, protein turnover, chaperones (121, 9.95%) (Figure 5).
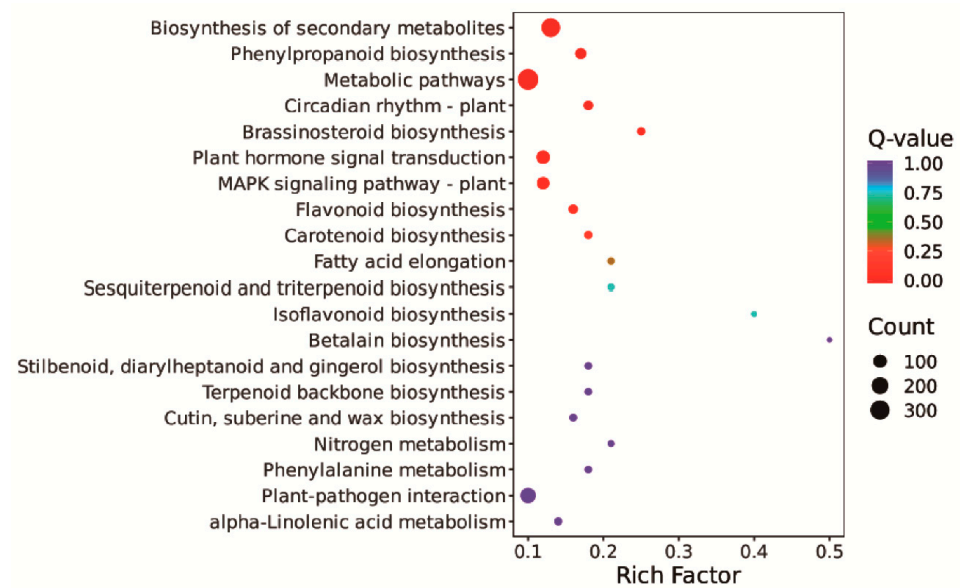
Figure 4. The KEGG enrichment analysis of DEGs of Songjing 2 rice under straw compost. The horizontal axis represented rich factor. The vertical axis represented the KEGG pathway. The larger the rich factor, the greater the enrichment level. The larger the dot, the more DEGs enriched in the pathway. The redder the dot color, the more significant the enrichment.
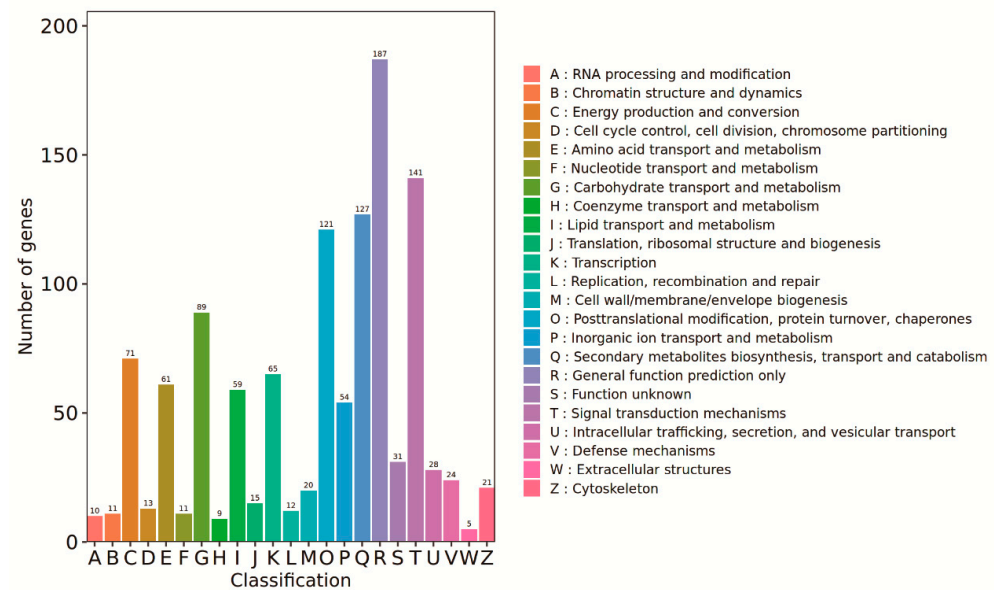

Figure 5. The KOG analysis of DEGs of Songjing 2 rice under straw compost. The horizontal axis represented the functional classification of KOG ID, the vertical axis represented the number of included DEGs, and different classifications were represented by different colors. The legend was a code with its functional description information.

3.6. qRT-PCR Verification of the RNA-Seq Results

To verify the RNA-seq data, eight significant DEGs were selected for a qRT-PCR analysis using gene-specific primers (Supplementary Table S2). The gene expression trends revealed by the qRT-PCR analysis were in accordance with the RNA-seq results (Figure 6), indicative of the reliability of the RNA-seq analysis.
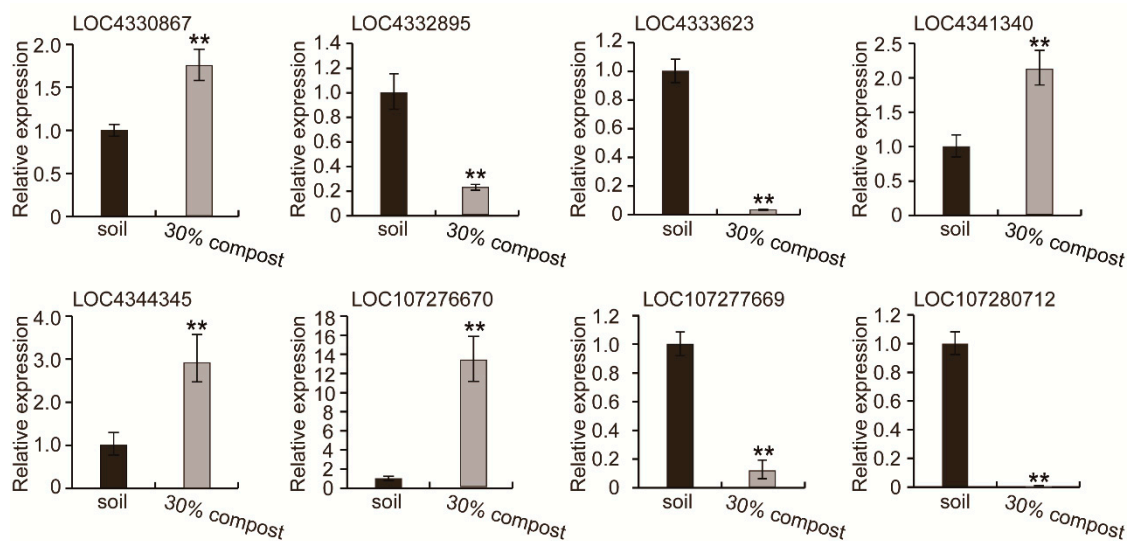
Figure 6. qRT-qPCR analysis of the DEGs. The mRNA levels of 8 randomly selected DEGs were measured by qRT-qPCR using rice *OseEF-1a* gene as an internal standard. The mRNAs of leaves under soil were used as controls. Data were shown as means ± SE of three independent experiments. Asterisks indicated statistically significant differences compared with the soil condition: ** $p < 0.01$ as determined by the Student's *t*-test.

4. Discussion

4.1. Utility of the Low-Temperature Fermented Straw Compost for Promoting Rice Growth and Increasing Yield

Straw is rich in nutrient elements; its decomposition can increase the soil organic matter content and fertility, which is critical for preventing farmland desertification and improving agricultural field conditions [16]. However, approximately 30% of straw cannot be fully decomposed under natural conditions because the polysaccharide components, such as cellulose, hemicellulose, and lignin, are difficult to degrade. Because the remaining straw is discarded or incinerated, straw resources are wasted and the environment is polluted [35]. The recent advances in microbial research have led to increasing interest in biological fermentation techniques involving the use of specific microorganisms for the efficient decomposition of materials. To date, *probiotics* [36], *Lactobacillus liquidarum* (type IV) [37], *C. rosea* strain YZC3 [38], and *G. stearothermophilus* [39] have been used as microbial starter cultures to effectively degrade straw. These microbes are suitable for fermenting straw at room temperature. The application of decomposed straw compost can increase the quality and yield of cucumber [36], potherb mustard [37], and other crops, with positive implications for sustainable agricultural production.

In China, considerable amounts of straw are produced annually in the northeastern region, which is the main grain-producing area. There are few fungi suitable for low-temperature fermentation. This has limited the low-temperature straw fermentation and the large-scale outdoor fermentation in northeastern China, which is relatively cold in the winter and spring. Therefore, developing viable methods for the efficient decomposition of straw under low-temperature conditions is crucial for optimizing the use of straw resources in northeastern China. Mu et al. recently isolated *Penicillium oxalicum* strain M11 from soil; the subsequent characterization of this strain indicated it can efficiently degrade cellulose at 13 °C [40]. In addition, Shao et al. isolated the cold-tolerant *Streptomyces* strain ND2-1 from forest soil samples collected in Daqinggou, Inner Mongolia; this strain can efficiently degrade cellulose at low temperatures and ferment straw [41]. Moreover, Li et al. observed that *Penicillium chrysogenum*, *Trichoderma citrinoviride*, and *Neurospora sitophila* can grow and degrade cellulose under cold conditions. When these fungi are combined, they can efficiently decompose waste straw at low temperatures in northeastern China [42]. Unfortunately, the effects of the resulting compost on crop growth and yield in northeastern

China have not been thoroughly investigated. In the current study, the effects of straw compost on the growth and yield of Songjing 2, which is a *japonica* rice cultivar grown in northern China, were investigated. Briefly, a microbial agent capable of composting straw at low temperatures was used to ferment and decompose waste straw, which was combined with Longmin black pig manure and then mixed with natural soil. Our analyses indicated that the straw compost-treated rice plants were significantly taller than the control plants. Moreover, the rice plant height increased significantly as the straw compost content in the soil increased (Figures 1A and S2). The straw compost treatment also significantly increased the rice root length as well as the 100-grain weight (Figure 1B,E). These results reflect the potential utility of this low-temperature straw fermentation technology in northeastern China and the broad applicability of straw composting technology to enhance agricultural production.

4.2. The Low-Temperature Fermented Straw Compost Promoted Rice Growth and Increased the Grain Yield by Altering Soil Physicochemical Properties and the Expression of Important Signaling Pathway Genes in Rice

Soil acidity/alkalinity is an important chemical property because of its effects on soil chemical reactions and nutrient availability for plant growth. The research has shown that the plant height, seed setting rate, and yield per plant decreased significantly when the soil pH was below 5.0 or above 7.0. However, these indices peaked when the soil pH was 6.0 [43]. The addition of straw compost caused the pH of the Songjing 2 rhizosphere soil to increase from 4.87 to 6.35, which is ideal for Songjing 2 rice plant growth and development (Table 3). Soil pH is influenced by various factors such as climate, terrain, and vegetation, etc. The effect of straw compost on soil pH is closely related to the initial pH of the soil. But, the main mechanism of soil acidification balance of weakly acidic soil caused by straw returning to the field has not been fully studied. However, the detailed reasons why straw compost treatment alleviates the acidification level of weakly acidic soil in this study are still unclear. We will further analyze this reason in depth in the future field application work of straw compost. Various soil enzymes have key functions in the soil ecosystem (e.g., material cycling and energy conversion) [44]. Invertase contributes to soil sucrose metabolism, which is closely related to soil fertility, microbial activity, and respiration [45]. Soil phosphatases help mediate organic phosphorus transformation and are associated with the formation of inorganic phosphorus. In addition, soil urease hydrolyzes urea to ammonia, while also regulating the nitrogen cycle [46]. These enzymes, which are useful indicators of farmland fertility, affect crop growth and development by modifying the soil microenvironment. In this study, the addition of straw compost significantly enhanced the urease, sucrase, and phosphatase activities in the rice rhizosphere (Table 4), which was conducive to rice growth. Soil organic matter can improve soil physicochemical properties and enhance soil fertility, thereby increasing crop yields [47]. Appropriate nitrogen, phosphorus, and potassium concentrations in the soil can promote plant growth and development. Our results showed that the straw compost treatment significantly increased the organic matter and total phosphorus contents in the rhizosphere soil of Songjing 2 (Table 3). As the active components of soil, microorganisms play an important role in material transport and nutrient cycling. Changes in soil microbial activities and community structures affect soil nutrient transformation, which in turn influences the growth, yield, and quality of crops [9]. The soil microbial abundance is influenced by various factors, including soil physicochemical properties and temperature, etc. Research has shown that the straw compost treatment can increase soil microbial abundance. But, in our study, the increase the number of fungi was very limited, and we consider whether this is due to potted crops causing less significant changes in fungal numbers. We will further investigate the impact of straw compost on soil microbial abundance in future field applications. We observed that the addition of straw compost increased the number of actinomycetes and bacteria in the soil. Notably, the number of actinomycetes was nearly four times higher in the straw compost-containing soil than in the control soil (Table 5). Therefore, the straw compost treatment increased the soil microbial richness, affected

the soil microbial community structure, and altered the transformation of soil nutrients, ultimately enhancing rice growth. In conclusion, the low-temperature fermented straw compost significantly improved various microenvironmental conditions (e.g., soil pH, enzyme activity, organic matter content, and microbial abundance), thereby promoting the growth of Songjing 2 rice plants. Because the soil colloidal particle size may be used as an indicator of soil quality [48], we will investigate whether straw composting influences the soil particle size as part of our future research.

In plants, stress-induced oxidation results in the production of malondialdehyde and other harmful substances [49]. However, the synthesis of antioxidants (e.g., SOD, POD, and CAT) can limit the toxic effects of malondialdehyde. Antioxidant enzymes convert peroxides into less toxic or harmless compounds. Therefore, antioxidant enzyme activities may reflect the ability of plants to decompose harmful substances [50]. In addition, soluble proteins are important osmoregulators and nutrients [34]. The accumulation of soluble proteins can positively affect the ability of cells to retain water, while also protecting specific cellular components and biofilms. The soluble proteins include the enzymes involved in various metabolism and physiological processes, which have very important roles. Our results showed the straw compost treatment increased stress resistance-related enzyme activities in Songjing 2 rice (Table 1). Chlorophyll absorbs solar energy and converts it to chemical energy during photosynthesis [19]. In response to the addition of straw compost, the chlorophyll contents in the rice leaves increased, which promoted photosynthetic activities, leading to increases in Songjing 2 rice growth and yield (Table 2). The DAB and NBT staining results showed that adding straw compost to the soil can decrease the H_2O_2 and superoxide anion contents in rice leaves (Figure 1C). We speculate that this may be the result of these stress-related enzymes activity being increased with straw compost treatment and playing a role. This speculation will be verified in future work. On the basis of these findings, we hypothesized that the application of straw compost can improve rice stress resistance. To test this hypothesis, we grew Songjing 2 rice plants in saline-alkali soil (pH 10.38) collected from the Anda region of northeastern China. The addition of straw compost to the soil significantly promoted plant growth, indicative of an increase in abiotic stress tolerance (Figure 7).
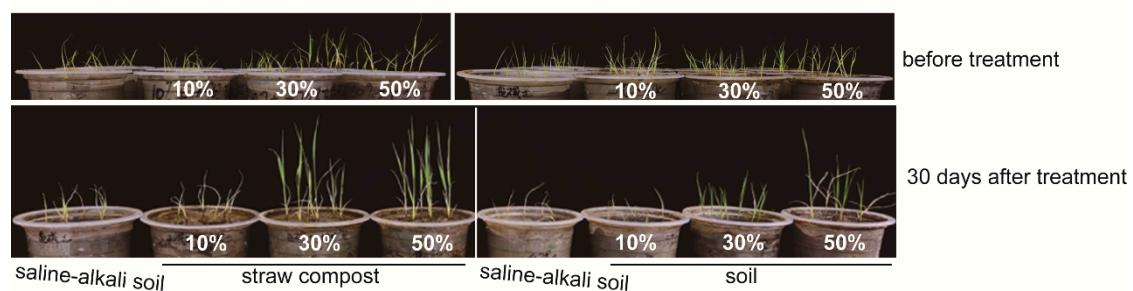

Figure 7. The effect of straw compost on Songjing 2 rice under salt alkali stress. The seedlings of Songjing 2 rice grown in normal soil for 14 days were transferred to saline alkali soil (pH10.38) and saline alkali soil with 10%, 30%, and 50% straw compost. After 30 days of planting, the growth status of the Songjing 2 rice was observed and photos were taken.

Recently, Chi et al. conducted an RNA-seq analysis and elucidated the molecular mechanism by which corn straw compost affects the growth and development of corn plants. Specifically, corn straw compost induces the accumulation of organic matter by promoting carbon metabolism and photosynthesis, which leads to enhanced corn plant growth [51]. There is currently very little available information regarding the molecular basis of the plant growth-promoting effects of straw compost. In this study, an RNA-seq analysis was conducted to examine the effects of straw compost in the soil on rice gene expression. A total of 2473 DEGs were identified (982 up-regulated genes and 1491 down-regulated genes) (Figure 2). The main significantly enriched GO terms assigned to the DEGs

suggested that the straw compost treatment affected the expression of genes associated with the signaling pathways influencing enzyme activities, metabolism and biosynthesis, and stress responses (Figure 3). Plant enzyme activities, metabolic and biosynthetic processes, and stress responses have crucial effects on crop growth and development. According to the results of the KEGG analysis, the DEGs encode proteins contributing to the metabolism and biosynthesis of secondary metabolites (Figure 4), indicating that straw composting mainly regulates the production of plant secondary metabolites, with implications for rice growth and yield.

In summary, the low-temperature fermented straw compost used in this study optimized the soil conditions for rice growth. More specifically, the application of straw compost improved the soil pH, increased the soil organic matter, total nitrogen, and total phosphorus contents, and increased soil enzyme activities. Furthermore, the straw compost treatment also significantly enriched the soil microbial community (i.e., bacteria and actinomycetes) and promoted the decomposition and transformation of organic materials by microorganisms, with positive effects on soil conditions. Additionally, the straw compost increased stress response-related enzyme activities and the chloroplast content in rice, while also modulating the expression of genes in metabolic pathways. These changes increased the growth and yield of rice plants (Figure 8). In future studies, we will more precisely analyze the molecular network underlying the regulatory effects of straw compost on rice growth and development by integrating metabolome and microbiome analyses. Moreover, rice mutants in which selected DEGs are silenced will be generated to further elucidate the molecular mechanism through which straw compost affects rice growth.
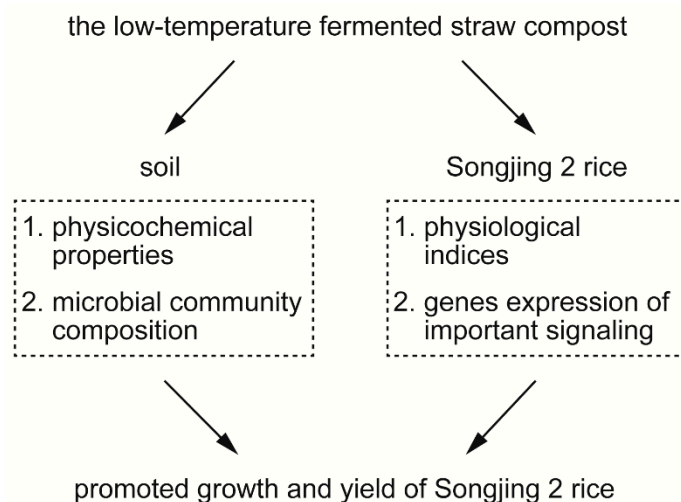

Figure 8. Possible model of the low-temperature fermented straw compost promoting Songjing 2 rice growth and increased yield. The low-temperature fermented straw compost promoted the Songjing 2 rice growth and increased yield by regulating soil physicochemical properties and the microbial abundance, as well as the physiological indices and genes expression of important signal pathway in Songjing 2 rice.

5. Conclusions

In this study, the addition of low-temperature fermented straw compost significantly improved soil physicochemical properties (e.g., pH, enzyme activity, and nutrient composition) and optimized the soil conditions for crop growth. The application of straw compost also increased soil microbial diversity and promoted the decomposition and transformation of organic matter, thereby improving the soil environment. The straw compost treatment also increased the rice leaf chlorophyll content and enzyme activities related to stress responses, which positively affected rice stress resistance. In addition, the application of straw compost influenced the expression of genes in rice metabolic pathways as well as pathways mediating secondary metabolite synthesis and plant hormone signal transduction. In

conclusion, the low-temperature fermented straw compost used in this study effectively improved the soil environment, increased the important physiological indices of Songjing 2, and affected the expression of critical signaling pathway genes, thereby promoting rice growth and increasing the grain yield. The study findings provide the theoretical basis for the commercial application of low-temperature straw fermentation technology to improve agricultural production.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13123066/s1, Figure S1: Collection of straw compost; Figure S2: Morphology of Songjing 2 rices grown for 3 months under soil and 30% straw compost conditions; Figure S3: Analysis of average GC content of transcriptome sequencing data; Figure S4: The GO enrichment analysis of DEGs under soil and straw compost; Figure S5: The KEGG enrichment analysis of DEGs that were expressed in soil and straw compost in Songjing 2 rice. Table S1: The proportion of straw compost in different conditions; Table S2: List of primers used for real-time PCR in this study; Table S3: List of DEGs identified from RNA-seq analysis.

Author Contributions: J.W., X.H. and Z.L. conceived and designed the experiments, and J.W., T.L. and Z.L. wrote the manuscript. T.L., K.X., N.Z. and Z.Z. performed the experiments. H.C., Y.F., X.H., W.W. and D.L. helped in the analysis of the results. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest: The authors declare that there are no conflict of interest.

References

1. Zhang, J.L.; Van Der Heijden, M.G.A.; Zhang, F.S.; Bender, S.F. Soil biodiversity and crop diversification are vital components of healthy soils and agricultural sustainability. *Front. Agric. Sci. Eng.* **2020**, *7*, 236–242. [CrossRef]
2. Rong, G.H. Experimental Study on Effects of Erosion and Tillage Measures on Soil Quality. Ph.D Thesis, Northwest A&F University, Xianyang, China, 2022.
3. Jacopo De Rosa, J.; Pontolillo, D.M.; Di Maio, C.; Vassallo, R. Chemical Clay Soil Improvement: From Laboratory to Field Test. *Procedia Eng.* **2016**, *158*, 284–289. [CrossRef]
4. Zhang, T.; Zhang, Y.X.; Xiang, H.Y. Research progress on effects and mechanisms of straw returning on soil fertility. *Jiangsu Agric. Sci.* **2018**, *46*, 22–28.
5. Wang, C.Q.; Xie, D.T.; Li, B.; Zhou, Y.; Li, H.X.; Li, T.X.; Zhang, X.Z. Effect of Different Kinds and Dosage of Organic Manure on the Yield and Quality of Celery. *Chin. Agric. Sci. Bull.* **2005**, *21*, 192–195.
6. Cao, G.L.; Zhang, X.Y.; Wang, D.; Zheng, F.C. Inventory of Emissions of Pollutants from Open Burning Crop Residue. *J. Agro-Environ. Sci.* **2005**, *4*, 800–804.
7. Hrynkiewicz, K.; Baum, C.; Leinweber, P. Density, metabolic activity, and identity of cultivable rhizosphere bacteria on *Salix viminalis* in disturbed arable and landfill soils. *Plant Nutr. Soil Sci.* **2010**, *173*, 747–756. [CrossRef]
8. Liu, J.P.; Ju, M.T.; Liu, Y.H.; Wang, P.; Wu, W.T.; Tong, S.M. The Analysis of China's Utilization Technology of Agricultural Straw and Development of Biomass Industry. *Ecol. Econ.* **2011**, *5*, 136–141.
9. Li, N.; Shao, T.Y.; Zhou, Y.J.; Cao, Y.C.; Hu, H.Y.; Sun, Q.K.; Long, X.H.; Yue, Y.; Gao, X.M.; Rengel, Z. Effects of planting *Melia azedarach* L. on soil properties and microbial community in saline-alkali soil. *Land Degrad. Dev.* **2021**, *32*, 2951–2961. [CrossRef]
10. Shi, Y. The Comprehensive Utilization of Crop Straw and in Binzhou Demonstration Application. Master's Thesis, Shandong University, Jinan, China, 2012.
11. Yin, J.; Liu, Y.; Yu, F.; Cai, J.; Liu, T. Screening and identification of a lignin degrading bacterium and its application in composting. *China Soil Fertil.* **2019**, *281*, 179–185.
12. Zhang, P.F.; Li, S.Y.; Yu, K.F.; Jiang, X.F. Screening of lignin-degrading bacteria and study on degradation of garden waste. *J. Anhui Agric. Univ.* **2018**, *45*, 676–681.
13. Wan, Y.; Liu, J.; Deng, F.; Xie, Z.J.; Chen, Y.C.; Li, J.B.; Li, D. Screening of lignin-degrading fungi and bioaugmentation on the directional humification of garden waste composting. *Ind. Crops Prod.* **2023**, *203*, 117208. [CrossRef]
14. Peng, X.G.; Yang, L.E.; Zhang, L. Study Progress on Biological Degrading of Lignin in Straws. *Xiandai Nongye Keji* **2010**, *1*, 18–20.
15. Kranz, C.N.; McLaughlin, R.A.; Johnson, A.; Miller, G.; Heitman, J.L. The effects of compost incorporation on soil physical properties in urban soils–A concise review. *J. Environ. Manag.* **2020**, *261*, 110209. [CrossRef] [PubMed]

16. Li, S.L.; Li, L.X.; Wang, Z.G.; Sun, J.; Zhang, H.L. Impacts of Corn Straw Compost on Rice Growth and Soil Microflora under Saline-Alkali Stress. *Agronomy* **2023**, *13*, 1525. [CrossRef]

17. Sarangi, S.; Swain, H.; Adak, T.; Bhattacharyya, P.; Mukherjee, A.K. Trichoderma-mediated rice straw compost promotes plant growth and imparts stress tolerance. *Environ. Sci. Pollut. Res.* **2021**, *28*, 44014–44027. [CrossRef] [PubMed]

18. Abbas, A.; Naveed, M.; Azeem, M.; Yaseen, M.; Ullah, R.; Alamri, S.; Ain, F.; Qurrat, U. Siddiqui Manzer H Efficiency of wheat straw biochar in combination with compost and biogas slurry for enhancing nutritional status and productivity of soil and plant. *Plants* **2020**, *9*, 1516. [CrossRef] [PubMed]

19. Pang, N.; Zhang, X.; Liu, J.Q.; Hu, Q.L.; Zhang, J.F. Study of compound microbial agents in the improvement of soda saline-alkali soil. *J. Jilin Agric. Univ.* **2023**, *9*, 1–12.

20. Li, F.L.; Xu, Y.Y.; Xu, Y.Q.; Feng, Y.Z.; Yang, X.M.; Yuan, Q.; Wang, L.J. Bacterial Strains of Low Temperature Fermentation of Corn Stover and Their Fermentation and Cultivation Methods and Applications. Chinese Patent CN108865927B, 6 April 2021.

21. Wang, H. Screening and Genetic Analysis of Saline-Alkali Tolerant Rice Varieties. Ph.D Thesis, Northeast Forestry University, Harbin, China, 2020.

22. Porra, R.J. The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls a and b. *Photosynth. Res.* **2002**, *73*, 149–156. [CrossRef]

23. Guo, L.F.; Zhang, X.C.; Zhao, J.W.; Zhang, A.Q.; Pang, Q.Y. Enhancement of sulfur metabolism and antioxidant machinery confers *Bacillus* sp. Jrh14-10-induced alkaline stress tolerance in plant. *Plant Physiol. Biochem.* **2023**, *203*, 108063. [CrossRef]

24. Ding, S.J.; Zhang, X.F.; Yang, W.L.; Xin, X.L.; Zhu, A.N.; Huang, S.M. Soil Nutrients and Aggregate Composition of Four Soils with Contrasting Textures in a Long-Term Experiment. *Eurasian Soil Sci.* **2021**, *54*, 1746–1755. [CrossRef]

25. Yang, L.; Tan, L.L.; Zhang, F.H.; Gale, W.J.; Cheng, Z.B.; Sang, W. Duration of continuous cropping with straw return affects the composition and structure of soil bacterial communities in cotton fields. *Can. J. Microbiol.* **2018**, *64*, 167–181. [CrossRef] [PubMed]

26. Shi, C.F.; Wang, Z.Y.; Leng, X.Y.; Liu, J.M.; Cao, J.M. Improvement of soil phosphatase activity measurement method. *Exp. Technol. Manag.* **2016**, *33*, 48–49+54.

27. Luo, M.X.; Hu, Z.D.; Liu, X.L.; Li, Y.F.; Hu, J.; Ou, D.H.; Wu, D.H. Characteristics of soil microbial biomass carbon, nitrogen and enzyme activities in Picea asperata plantations with different ages in subalpine of western Sichuan, China. *Acta Ecol. Sin.* **2021**, *41*, 5632–5642.

28. Yu, J.L.; Shi, H.X. Changes of Microbes Population in the Different Degraded Alpine Meadows on the Qinghai-Tibetan Plateau. *Acta Agric. Boreali-Occident. Sin.* **2011**, *20*, 77–81.

29. Chen, S.; Zhou, Y.; Chen, Y.; Gu, J. fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* **2018**, *34*, i884–i890. [CrossRef] [PubMed]

30. Kim, D.; Langmead, B.; Salzberg, S.L. HISAT: A fast spliced aligner with low memory requirements. *Nat. Methods* **2015**, *4*, 357–360. [CrossRef] [PubMed]

31. Varet, H.; Brillet-Guéguen, L.; Coppée, J.Y.; Dillies, M.A. SARTools: A DESeq2- and EdgeR-Based R Pipeline for Comprehensive Differential Analysis of RNA-Seq Data. *PLoS ONE* **2016**, *11*, e0157022. [CrossRef]

32. Xie, Q.J.; Liu, B.C.; Dong, W.F.; Li, J.H.; Wang, D.N.; Liu, Z.Y.; Gao, C.Q. Comparative transcriptomic and metabolomic analyses provide insights into the responses to NaCl and Cd stress in *Tamarix hispida*. *Sci. Total Environ.* **2023**, *884*, 163889. [CrossRef]

33. Chen, S.; Huang, D.; Zhang, L.; Guo, X.P.; Zhang, S.X.; Cao, X.C. The Effects of Straw Incorporation on Physicochemical Properties of Soil: A Review. *J. Irrig. Drain.* **2022**, *41*, 1–11.

34. Wang, Z. Effects of Compound Microbial Agents on the Growth and Development of Rice. Master's Thesis, Shenyang Agricultural University, Shenyang, China, 2017.

35. Li, J.; Li, M.; Gao, X.X.; Fang, F. Corn straw mulching affects *Parthenium hysterophorus* and rhizosphere organisms. *Crop Prot.* **2018**, *113*, 90–96. [CrossRef]

36. Yu, Z.Q.; Xu, Y.Q.; Li, F.L.; Liu, D.; Hao, L.B.; Wang, M.J.; Wang, X.; Dong, S.J.; Hu, B.Z. Effects of Composting Mulch and Organic Fertilizer Fermented by EM on Cucumber Quality. *Crops* **2015**, *3*, 104–110.

37. Chen, J.J.; Zhang, T. Effects of banana stalk and leaf straw fertilizer on the growth and quality of mustard. *Shanghai Veg.* **2022**, *3*, 42–44.

38. Liang, X.; Huang, C.Z.; Deng, Z.W.; Liang, A.; Yu, T. Study on the application effect of organic material decomposition agent in returning banana stems and leaves to field. *Agric. Technol.* **2017**, *37*, 59–61.

39. Ding, Z.L.; Han, L.N.; Zeng, H.C.; Zheng, W.; He, Y.D.; Zang, X.P. Effect of Banana Stalk Organic Fertilizer on Chinese Cabbage Growth. *Chin. Agric. Sci. Bull.* **2016**, *32*, 73–78.

40. Mu, C.L.; Wu, X.S.; Li, S.N.; Ma, M.C.; Li, J.; Shen, B.L.; Zhu, B.C. Screening and identification of a cold-adapted cellulase-producing strains and characterization of cellulose. *Acta Microbiol. Sin.* **2013**, *40*, 1193–1201.

41. Shao, N.N.; Xuan, H.L.; Luo, F.; Dai, X.Z. Isolation, identification and characterization of a cold-adapted cellulaseprodu Cing psychrotrophic *Streptomyces* sp. *Sci. Technol. Food Ind.* **2015**, *36*, 159–163.

42. Feng, X.X.; Li, F.L.; Xu, Y.Q. Screening of cellulase producing strains from rotten wood in Xinjiang cold area and analysis of their characteristics of enzyme production at low temperature. *Acta Agric. Zhejiangensis* **2021**, *33*, 1468–1476.

43. Yi, Y.K.; Zhou, Z.B.; Chen, G.H. Biomass-based dynamic model for rice root system. *J. Agro-Environ. Sci.* **2017**, *36*, 428–436.

44. Bailey, V.L.; Fansler, S.J.; Smith, J.L.; Bolton, H. Reconciling apparent variability in effects of biocharamendment on soil enzyme activities by assay optimization. *Soil Biol. Biochem.* **2011**, *43*, 296–301. [CrossRef]

45. Mendes, R.; Kruijt, M.; De Bruijn, I.; Dekkers, E.; Van der Voort, M.; Schneider, J.H.; Piceno, Y.M.; DeSantis, T.Z.; Andersen, G.L.; Bakker, P.A.; et al. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science* **2011**, *332*, 1097–1100. [CrossRef]
46. Wang, L.; Pang, X.; Li, N.; Qi, K.; Yin, C. Effects of vegetation type, fine and coarse roots on soil microbial communities and enzyme activities in eastern Tibetan plateau. *Catena* **2020**, *194*, 104694. [CrossRef]
47. Sun, H.Y.; Li, X.K.; Ren, T.; Cong, R.H.; Lu, J.W. Effects of Fertilizer in Shallow Soils on Growth and Distribution of Rice Roots at Seedling Stage. *Sci. Agric. Sin.* **2014**, *47*, 2476–2484.
48. Cogger, C.G. Potential compost benefits for restoration of soils disturbed by urban development. *Compos. Sci. Util.* **2005**, *13*, 243–251. [CrossRef]
49. Abdelaziz, M.; Xuan, T.; Mekawy, A.; Wang, H.; Khanh, T. Relationship of Salinity Tolerance to Na^+ Exclusion, Proline Accumulation, and Antioxidant Enzyme Activity in Rice Seedlings. *Agriculture* **2020**, *8*, 166. [CrossRef]
50. Kibria, M.G.; Hossain, M.; Murata, Y.; Hoque, M.A. Antioxidant Defense Mechanisms of Salinity Tolerance in Rice Genotypes. *Rice Sci.* **2017**, *24*, 155–162. [CrossRef]
51. Chi, K.W. Effects of Maize Straw Composting on Maize Growth and Development and Transcriptome Analysis under Saline-Alkali Stress. Master's Thesis, Northeast Forestry University, Harbin, China, 2021.