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Effect of Low-Temperature-Fermented Straw Compost on Alfalfa (*Medicago sativa* L.) Growth and Stress Tolerance

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Abstract

The physicochemical properties of soil have a significant impact on plant growth, and fertilizers that improve soil quality play a crucial role in promoting crop development. Abandoned crop straw, once composted and converted into organic fertilizer, is an important resource for agricultural production. However, the mechanisms by which straw compost regulates plant growth remain incompletely understood. In previous work, we demonstrated that low-temperature-fermented straw compost significantly promoted the growth and yield of rice. To further investigate the effects of low-temperature-fermented straw compost on alfalfa growth, this study incorporated 10% and 30% straw compost into soil for alfalfa cultivation and systematically compared plant growth and soil quality indicators. Results showed that compost application increased alfalfa leaf length by 30.55%, with significant improvements observed in multiple physiological parameters. Furthermore, straw compost amendment raised soil pH to 6.88, substantially enhanced soil organic matter content, and increased the activities of sucrase and urease by approximately 181.77% and 223.81%. The abundance of soil microorganisms, including fungi and actinomycetes, increased by approximately 129.59% and 444.44%, respectively, indicating that straw compost effectively improves soil conditions and promotes alfalfa growth. Overall, the study demonstrates that low-temperature-fermented straw compost exerts a promoting effect on crop growth and provides an important theoretical basis for promoting low-temperature straw fermentation technology and the application of straw composting in agricultural and animal husbandry production.

Keywords: straw compost; alfalfa; plant physiological indicators; transcriptome sequencing; soil physicochemical properties; soil microbial diversity

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1. Introduction

Soil is a vital component of agricultural resources, supporting a wide range of production activities. However, with the intensification of agricultural practices and improper land management, soil fertility gradually declines, reducing its capacity to sustain crop growth. This deterioration can lead to lower yields or even crop failure, thereby threatening agricultural production. Improving soil quality to enhance crop yields is therefore crucial for meeting food demands. Traditional approaches to soil improvement include

physical and chemical methods. Chemical methods typically rely on inorganic fertilizers to boost production, but the excessive use of such fertilizers has damaged soil ecosystems, undermining the sustainability of agricultural development [1].

Straw, the primary by-product of crop harvests, contains essential nutrients such as organic carbon, nitrogen, phosphorus, and potassium [2]. When properly managed and converted into renewable resources, straw plays a significant role in improving crop yields. Nevertheless, because it is rich in difficult-to-degrade components like cellulose and lignin, the conventional practice of directly returning straw to the field leaves approximately 30% undecomposed. This not only wastes resources but also contributes to environmental pollution [3]. For these reasons, developing efficient and environmentally friendly technologies for straw treatment is of considerable importance.

Composting technology is a biological treatment method that relies on microorganisms to decompose organic waste (such as straw, kitchen waste, etc.) and convert it into organic fertilizer [4]. Research has shown that many microorganisms play a crucial role in straw composting. For example, the fungal strains MSDA1 and HDGA2 possess lignin peroxidase and manganese peroxidase activities, enabling them to effectively degrade lignin and making them suitable for composting garden waste [5]. Similarly, the bacterial strain *1EJ7* can produce cellulase to break down straw, aiding in composting and maturation [6]. More recently, microorganisms isolated from low-temperature environments have been found to degrade straw effectively. For instance, *Sphingobacterium* spp. [7], *actinomycetes* ND2-1 [8], and *Pseudomonas psychrophila* BYAU-6 [9] can degrade lignocellulose under low-temperature and even freezing conditions, thereby accelerating straw residue decomposition in cold soils. The discovery of these cold-tolerant fungi has been particularly important for promoting straw degradation and composting fermentation in colder regions. The composite microbial agent M44, composed of dominant bacteria such as *Pseudomonas*, *Brevundimonas,* and *Flavobacterium*, regulates the structure of the soil microbial community and optimizes microbial interactions. This enhances the activity of enzymes involved in cellulose degradation, thereby significantly increasing the degradation rates of cellulose, hemicellulose, and lignin, and improving the overall decomposition efficiency of straw under low-temperature conditions [10].

Compost derived from straw through microbial degradation improves soil quality and fertility and is considered a beneficial ecological organic fertilizer that has been tested in agricultural production. The application of corn straw compost reduced soil ammonium nitrogen and nitrate nitrogen, increased soil microbial biomass carbon and nitrogen as well as organic carbon, and significantly improved wheat yield [11]. The addition of vegetable straw compost increased vitamin C, soluble protein, and soluble sugar in spinach, thereby improving spinach quality [12]. Under saline-alkali conditions, applying straw compost in combination with nano zinc oxide and gypsum effectively improved soil quality, promoted nutrient uptake in wheat, and increased wheat yield [13]. The combined application of fruit and vegetable compost with biochar significantly increased soil carbon, phosphorus, and potassium contents and the number of microorganisms; enhanced the activities of soil urease and β-glucosidase; and effectively improved corn plant height and thousand-grain weight [14]. Composting significantly enhanced soil nutrients and the corresponding soil enzyme activity, improved leaf photosynthetic traits and ion homeostasis, and also reduced the relative abundance of pathogenic fungi, thereby significantly increasing the leaf area, leaf nitrogen content and leaf water content of alfalfa seedlings, promoting the growth of alfalfa [15].

Taken together, these results indicate that applying straw compost effectively improves the physical and chemical properties of soil and increases crop quality and yield, although research on its mechanisms of action remains relatively limited. Previously, we confirmed

that low-temperature-fermented straw compost promotes rice growth and fruiting by influencing soil physicochemical properties, rice physiological indicators, and the expression of important signal genes [16]. Alfalfa is a perennial leguminous crop that is rich in protein, various vitamins, saponins, flavonoids, and other essential nutrients and bioactive compounds. It can be processed into a wide range of feed products and plays a significant role in animal husbandry production [17]. Therefore, alfalfa was chosen as the research subject. This study focused on the microbial-mediated low-temperature fermentation of straw compost and investigated the effects of straw compost on the growth and development of alfalfa, as well as the physical and chemical properties of the soil. The use of straw compost significantly promoted the growth of alfalfa, and also improved various plant physiological indicators. Moreover, the application of straw compost enhanced the quality of the soil. And provides an important theoretical basis for promoting low-temperature straw fermentation technology and the application of straw composting in agricultural and animal husbandry production.

2. Materials and Methods

2.1. Experimental Materials

The straw compost and soil from the Animal Husbandry Innovation Base of the Institute of Animal Husbandry, Heilongjiang Academy of Agricultural Sciences (45°40′ N, 126°33′ E) were used in this study. Straw compost for subsequent experiments was obtained by using microbial preparations for straw decomposition in cold areas, adding black pig manure, and fermenting and decomposing straw for about 150 days, and impurities such as plastic bags and stones were removed (Figure S1) [18]. 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. The treatment group consisted of a mixture of soil with straw compost at a ratio of 10%, 30% (*v*/*v*) thoroughly until it was evenly combined, while the control group consisted of only normal soil (Table 1) [19]. Other fertilizers were not used during the cultivation.

Table 1. The proportion of straw compost in different conditions.

Mixture Components
0% straw compost + 100% soil (control)
10% straw compost + 90% soil
30% straw compost + 70% soil

The alfalfa (*Medicago sativa* L. cv Longdong) was provided by the Institute of Cultivation, Heilongjiang Academy of Agricultural Sciences. *M. sativa* cv Longdong is a commonly sown cultivar in semi-arid regions of northern China. In the experiment, 30 alfalfa seeds from the same batch were soaked in 10% NaCIO for 2 h, soaked in 75% alcohol for 15 min, washed 5 times with sterile water [20]. After sterilization, the seeds were inoculated onto 1/2MS medium and subjected to vernalization for 3 days. Then, they were cultivated in a 26 °C plant growth chamber for another 3 days. Similar-sized seedlings were selected and planted in plastic buckets (upper diameter × lower diameter × height = 11.7 cm × 7.8 cm × 9.2 cm), with 4 seedlings per bucket, spaced 8 cm apart. Each bucket was filled with 424 g of soil. Alfalfa was planted in the 26 °C plant growth chamber from early March to early May 2023. The cultivation light conditions were 16 h of bright light and 8 h of darkness. During the entire growth period, 300 mL of water was applied every 2 days. And various biomass parameters, including plant height, leaf length, and leaf water loss, were analyzed. The test was conducted on the campus of Northeast Forestry University

in Harbin, Heilongjiang Province, China (47°16′ N, 123°55′ E). Each treatment comprised three replicates, and the entire experiment was repeated three times (2023–2025).

2.2. Determination of Physiological Indicators of Alfalfa

According to the instructions provided by the kit (Nanjing Institute of Construction Bioengineering, Nanjing, China), about 0.1 g of fresh alfalfa leaf samples during the growth period under different growth conditions were used to determine physiological indexes. The soluble protein content, activities of stress response-related catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) and superoxide dismutase (SOD) activities were evaluated, and the content of hydrogen peroxide (H_2O_2) was detected [21]. The effect of straw composting on chlorophyll content in leaf plants was evaluated according to Porra [22].

2.3. DAB and NBT Staining of Alfalfa Leaves

Healthy, uniformly sized three alfalfa leaves were incubated in DAB staining solution (containing 0.1 mg DAB, 1 mL 0.05 M Tris-HAC, pH 5.0) and NBT staining solution (containing 0.5 mg NBT, 1 mL 25 mM HEPES, pH 7.6) in the dark for approximately 24 h, the staining solutions were removed, and bleaching solution (95% ethanol) was used for boiling decolorization, and photos were taken [23].

2.4. Determination of Alfalfa Blade Length and Leaf Water Loss Rate

From the control group and the experimental group, 8 leaves were randomly selected from the same part of the stems of 12 plants that had grown for 2 months. The lengths of these leaves were compared using the ImageJ (v1.54f) software (https://imagej.nih.gov/ij/download.html, accessed on 10 October 2025). At the same time, using the same method, 5 leaves were selected, and the water loss rate of the leaves was measured with an electronic scale. All experiments were performed with three biological replicates.

2.5. Determination of Soil Physiochemical Properties

Soil samples from the rhizosphere of alfalfa were collected, and their physical and chemical properties were determined. Soil samples were completely saturated with water at a ratio of 1:2.5 (w/v) and centrifuged for 15 min at room temperature to obtain a soil-water suspension [19]. The acidity of the saturated soil extract in the soil-water suspension was determined using a pH meter (DF-808A, Guangzhou Dengfeng Analytical Instrument Factory, Guangzhou, China).

The Kjeldahl method was used to determine the hydrolyzable nitrogen (N) content in soil [24]. After humidification, the content of available phosphorus (P) in the soil was determined by the molybdenum blue colorimetric method [25]. Available potassium (K) content in soil was determined by extraction with neutral ammonium acetate solution and flame photometry [26]. All results were calculated based on dry weight at 105 °C [27].

The sucrase activity in soil was determined by 3,5-dinitrosalicylic acid colorimetric method at 508 nm. Phosphatase activity in soil was detected by the colorimetric assay using disodium phenyl phosphate as substrate and measured using a spectrophotometrically at 660 nm [28]. Catalase activity in soil was detected by potassium permanganate titration.

Bacteria, fungi and actinomycetes were determined by plate coating and counting method. Samples were taken from the soil at a height of 6–7 cm from the surface. The rhizosphere soil was obtained by shaking the alfalfa roots, and then it was quickly sieved and naturally air-dried to obtain the alfalfa rhizosphere soil. Potato sucrose medium was used to determine fungi, beef extract peptone medium was used to determine bacteria, and Gao's medium No. 1 was used to determine actinomycetes [29]. The prepared soil diluent was coated and cultured on the plate. Bacteria and actinomycetes were inverted and cultured at 28 °C for 7–10 days, and fungi were cultured at 25 °C for 3–5 days. Microscopic examination

and counting were carried out, and the number of soil microorganisms (CFU/g) was calculated according to the formula: soil microorganism number (CFU/g) = (average colony number × dilution ratio) ÷ soil dry weight [29].

2.6. Alfalfa Transcriptome Sequencing and Data Analysis

Select healthy and equal-sized alfalfa leaves from the control group and the 30% treatment group that have grown for two months. Take 0.1 g from each group and conduct liquid nitrogen grinding. Then extract total RNA from each sample. The cDNA libraries used for transcriptome sequencing were constructed and sequenced by Figshare. After the RNA sample was identified, the mRNA was first isolated, the cDNA was synthesized, and then the entire library was prepared by end repair and addition of A-tail. DNB (DNA Nano Ball) is then prepared, which is then loaded onto a sequencing chip and sequenced using a high-throughput sequencer. Once the library was built, it was used for initial quantification, and then the inserts of the library were detected using the Agilent 2100 and then inspected. Raw data was filtered using fastp (v0.23.4) [30], and then high-quality reads were mapped to the genome using HISAT [31]. Differential expression between the two groups was analyzed using DESeq2 (v1.48.1) [32], meeting $|\text{Log2Fold Changes}| \geq 1$ and FDR < 0.05 were identified as differentially expressed genes (DEGs). KEGGs, GOs, and KOGs were annotated, and enrichment analyses were performed using cluster analyzers.

2.7. RT-qPCR Analysis

Total RNA from alfalfa leaves was extracted using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). cDNA was prepared from 6 μg of total RNA under different conditions using the Prime Script RT Kit with gDNA Eraser (Takara Bio, Kusatsu, Shiga, Japan). Next, 40 ng cDNA and 40 nM primers were used for each RT-qPCR reaction using 2× Brilliant III SYBR Green QPCR Master Mix (Agilent, Santa Clara, CA, USA) and an Mx3000P Real-Time Thermal Cycling System (Agilent, Santa Clara, CA, USA) (Table S7). Each expression level was normalized with the 18S rRNA gene [33].

2.8. Statistical Analysis

The data obtained in this study are the average values of three repeated experiments. The data were analyzed using one-way ANOVA in SPSS (v21.0), and the statistically significant differences were calculated based on Student's *t*-test, with $p < 0.05$ (*) and $p < 0.01$ (**) as the significance thresholds.

3. Results

3.1. Straw Compost Influences the Growth of Alfalfa

Our previous research has revealed the significant importance of low-temperature-fermented straw compost in enhancing growth and yield of rice. Here, we investigated the impact of straw compost on alfalfa. The control plants and those treated with 10% and 30% straw compost were similar in height during the first 7 days of growth under different planting conditions, and there were no significant differences in the morphological characteristics of alfalfa (Figure S2). However, after 2 months of growth, the plants treated with straw compost were significantly taller than the control plants (Figure 1A,B). Specifically, the root system of the plants treated with 30% straw compost was significantly longer than that of the control plants (Figure 1B), and the leaves of the plants treated with 30% straw compost were significantly longer than those of the control plants (Figure 1C, Tables S1 and S8). Meanwhile, the water loss from the leaves of alfalfa plants treated with 30% straw compost was significantly lower than that of control alfalfa plants (Figure 1D).

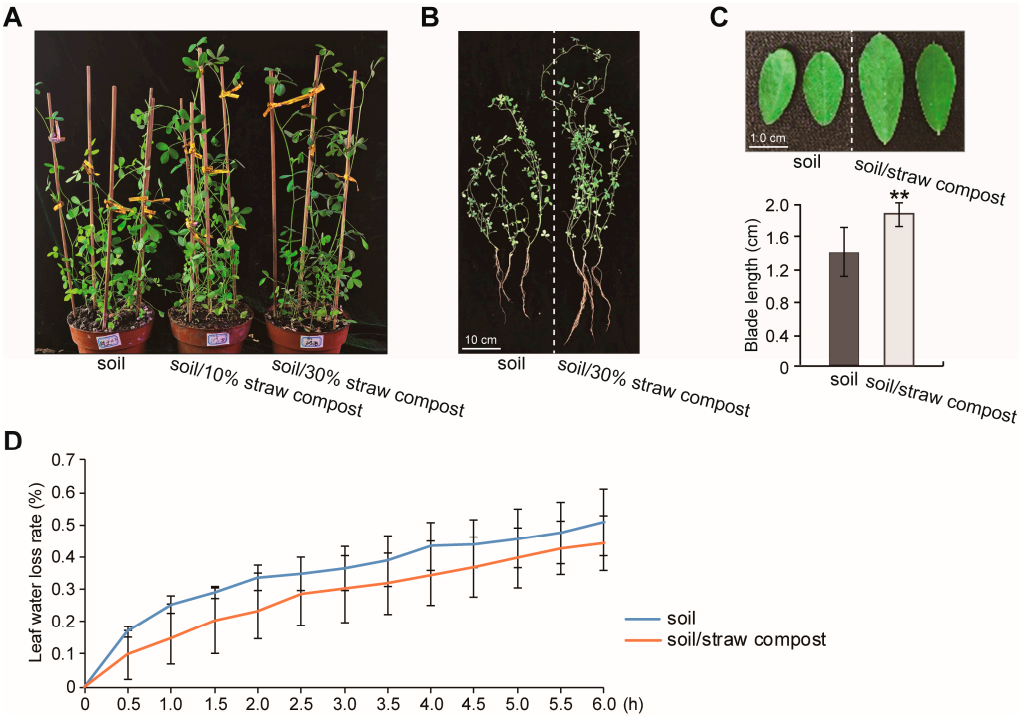


Figure 1. Effect of straw composting on alfalfa growth. (**A**) Alfalfa seedlings grown in normal soil for 7 days were transferred to normal soil and normal soil with 10% and 30% straw compost growing for 2 months, the alfalfa growth status was observed and photographed. (**B**) The morphology of alfalfa grown for two months in normal soil and normal soil with 30% straw compost. (**C**) Comparative analysis of length of alfalfa leaf from normal soil and normal soil with 30% straw compost. Each data value represented the mean ± SE of three replicates, with each using 8 alfalfa leaves. Asterisks indicate statistically significant differences compared with the normal soil: ** $p < 0.01$, as determined by Student's *t*-test. (**D**) The water-loss rate of alfalfa leaves in normal soil and normal soil with 30% straw compost. Each data value represented the mean ± SE of three replicates, with each using 5 alfalfa leaves.

3.2. Effects of Straw Compost on Various Physiological Indicators of Alfalfa

Previous studies have shown that the application of straw compost can affect various physical and chemical properties of crops [34]. Therefore, we further analyzed the effects of low-temperature-fermented straw compost on various physiological indicators of alfalfa. The results showed the POD activity of plants treated with 30% straw compost was approximately 1.5 times that of the control plants (Figure 2A, Tables S2 and S8). Similarly, CAT, a key enzyme for decomposing H_2O_2, had an activity more than twice that of the control plants (Figure 2A, Tables S2 and S8). APX and SOD are both important antioxidant enzymes, and their activities have also been enhanced (Figure 2A, Tables S2 and S8), and H_2O_2 content in leaves was significantly reduced (Figure 2B, Tables S2 and S8). Therefore, straw compost treatment significantly enhanced the activities of enzymes related to alfalfa's stress response, thereby increasing its stress resistance.

The DAB staining results showed that the brown spots on the leaves of the control plants were significantly darker than those on the leaves of the plants treated with straw compost (Figure 2C). Similarly, in the NBT-stained samples, the blue spots on the leaves of the control plants were deeper than those on the leaves of the plants treated with straw compost (Figure 2C).

The chlorophyll a, b and carotenoid contents in the leaves treated with 30% straw compost were higher than those of the control plants (Figure 2D, Tables S3 and S8), respectively.

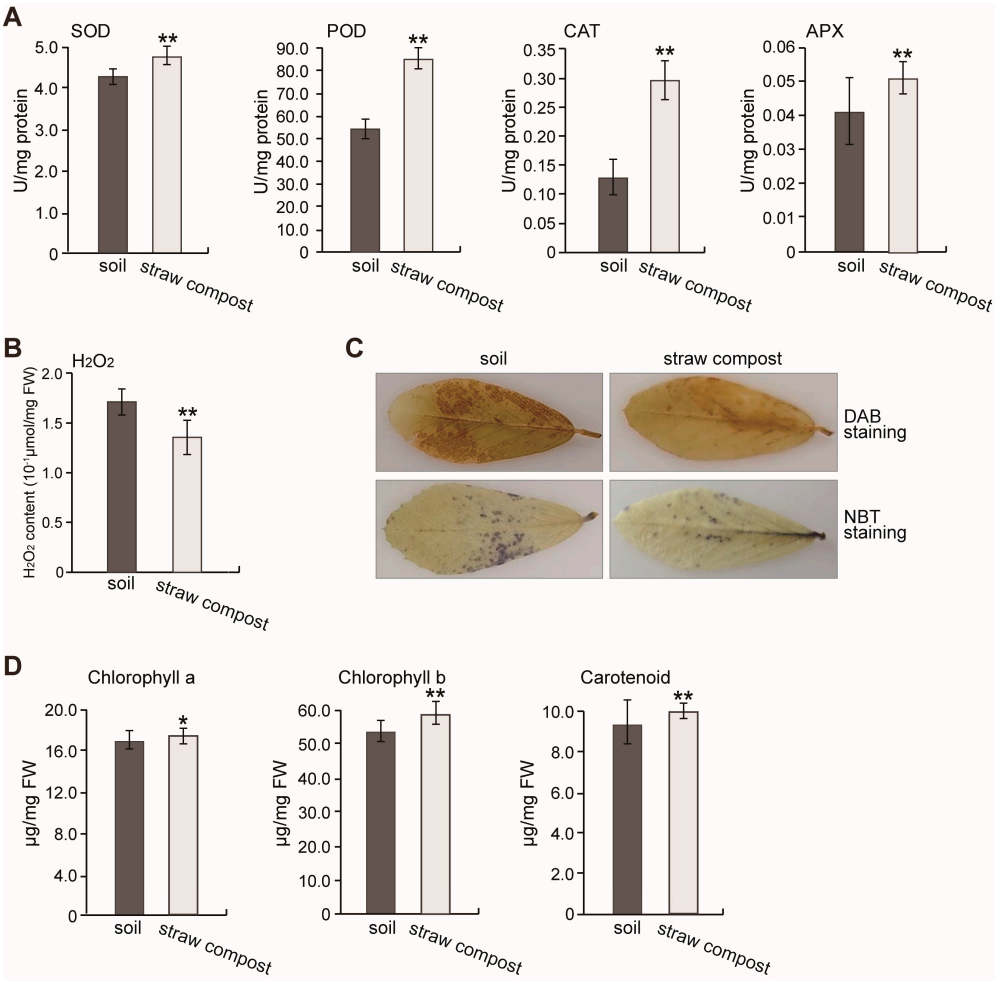


Figure 2. The impact of straw compost on various physiological indicators of alfalfa grown for two months in normal soil and normal soil with 30% straw compost. (**A**) The analysis of activities of stress response-related enzymes of alfalfa. (**B**) Determination of H_2O_2 content of alfalfa. Each data value represented the mean ± SE of three replicates, asterisks indicated statistically significant differences compared with the normal soil: * $p < 0.05$ and ** $p < 0.01$ as determined by Student's *t*-test. (**C**) NBT and DAB staining of alfalfa leaves. (**D**) Determination of the chlorophyll content in alfalfa leaves.

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

The contents of available P and available K in the soil with 30% straw compost were significantly higher than those in the soil control, and the content of hydrolyzable nitrogen (N) was slightly higher than that of the soil control (Figure 3A, Tables S4 and S8). The pH of the soil with 30% straw compost (6.88) was higher than that of the control soil (6.48) (Figure 3B, Tables S4 and S8).

In soil supplemented with 30% straw compost, sucrase and urease activity increased nearly threefold compared to the control group, respectively; the catalase activity did not change much, but phosphatase activity decreased slightly. These results suggest that the application of straw compost can improve the physical and chemical properties of the soil (Figure 3C, Tables S5 and S8).

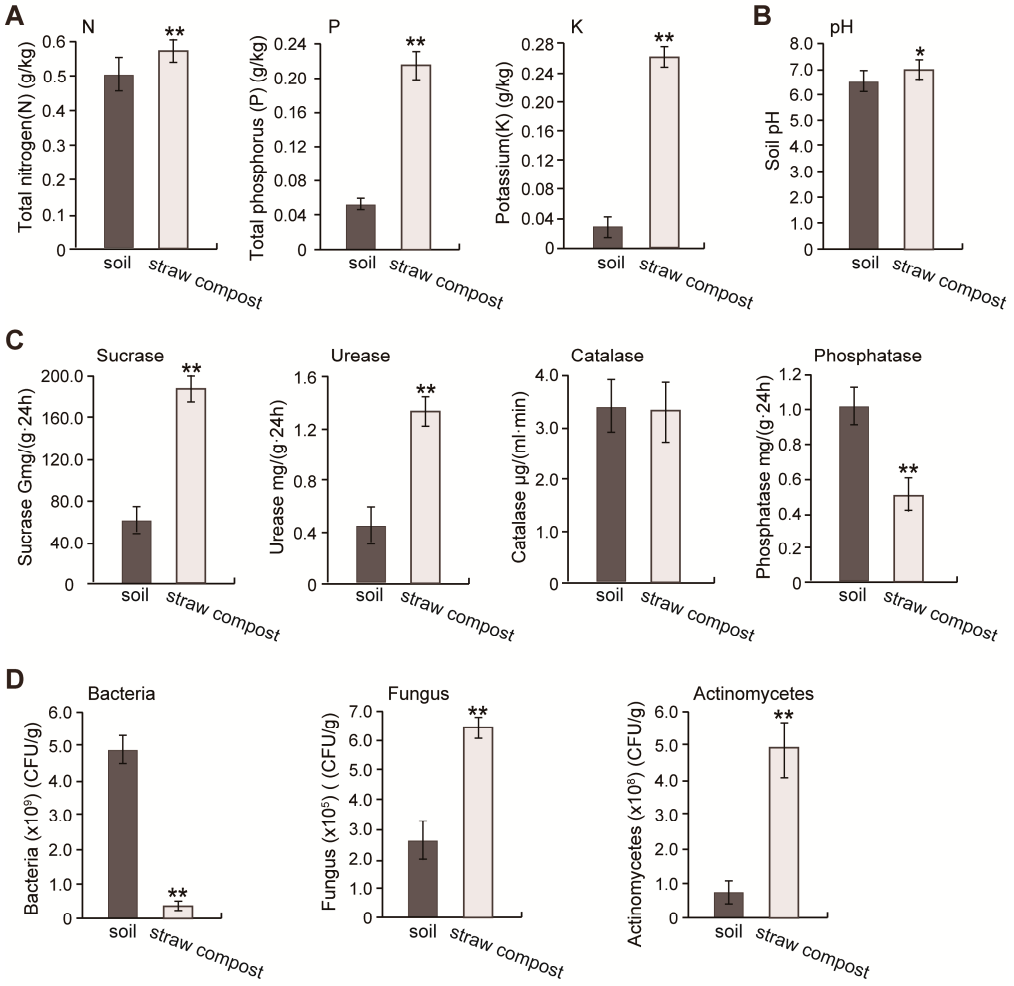


Figure 3. The effect of straw compost on alfalfa rhizosphere soil. (**A**) The analysis of organic matter contents of soil. (**B**) The detection of soil pH. (**C**) The analysis of soil enzyme activities. (**D**) The determination of soil microbial abundance. Each data value represented the mean ± SE of three replicates, asterisks indicated statistically significant differences compared with the normal soil: * $p < 0.05$ and ** $p < 0.01$ as determined by Student's *t*-test.

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

The abundance of actinomycetes in the soil supplemented with 30% of the straw compost was more than five times that of the control soil (Figure 3D, Tables S6 and S8). The quantity of fungi in the soil containing 30% of the straw compost was more than twice that of the control soil (Figure 3D, Tables S6 and S8). However, the number of bacteria was less than that of the control group (Figure 3D, Tables S6 and S8). Overall, the addition of straw compost increased the richness of soil microorganisms.

3.5. Reprograms in the Alfalfa Leaf Transcriptome in Response to the Straw Compost Application

High-quality reads were obtained, with an average GC content of 43.4% (Figure S3), indicating that the data obtained were suitable for subsequent analysis. Principal component analysis (PCA) distinguished the two experimental groups, with a value of principal component 1 (PC1) of 32.86% and that of PC2 of 20.78% (Figure 4A). A total of 2178 genes were differentially expressed between control alfalfa and straw compost-treated plants. Of these DEGs, 796 and 1382 had up- and down-regulated expression levels, respectively, in straw-composted plants (Figure 4B and Table S9).

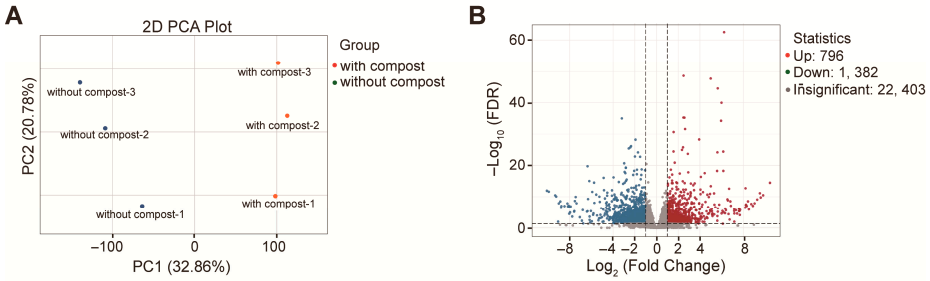


Figure 4. DEGs analysis of alfalfa using transcriptome sequencing data under straw composting. (**A**) Principal component analysis of DEGs. Horizontal axis (PC1): represents the most obvious feature describing the multidimensional data matrix. Vertical axis (PC2): represents the most significant feature of the data matrix beyond PC1. (**B**) Volcano plot of alfalfa DEGs under straw compost. The horizontal axis shows the change in gene expression fold, and the vertical axis shows the significance level of DEGs. Red dots indicate up-regulated DEGs, blue dots indicate down-regulated DEGs, and gray dots indicate non-DEGs.

The significantly enriched GO terms in DEGs include response to salicylic acid, response to hypoxia, response to decreased oxygen levels, response to oxygen levels, as well as other GO terms such as response to wounding, stomatal movement, regulation of response to biotic stimulus, regulation of response to external stimulus, plant organ senescence, and secondary metabolite biosynthetic process (Figure 5 and Figure S4, Table S10). Therefore, the DEGs are associated with enzyme activity, metabolism and biosynthesis, as well as responses to stress.

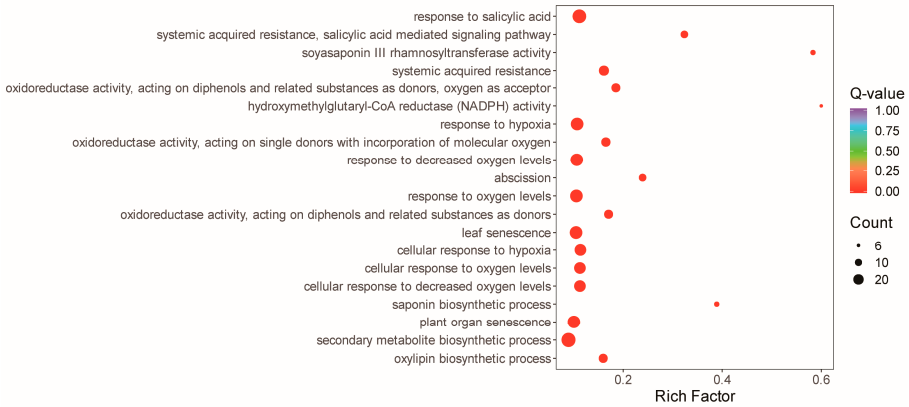


Figure 5. GO enrichment analysis of alfalfa DEGs under straw composting. The vertical axis represented the GO entries, and the horizontal axis represented the rich factor. The larger the rich factor, the greater the degree of enrichment. The larger the dot, the more DEGs were in the GO entry. The redder the color of the dot, the more significant the enrichment was.

DEGs were subjected to a KEGG (Kyoto Encyclopedia of Genes and Genomes) analysis. The results showed that 107 genes were involved in plant hormone signaling (KO04075), 256 genes were involved in the Metabolic pathways (KO01100), 169 genes were involved in the biosynthesis of secondary metabolites (KO01110), and 173 genes were involved in plant-pathogen interaction (KO04626). Based on the KEGG analysis results, these DEGs are particularly abundant in the metabolism of secondary metabolites and plant-pathogen interaction pathways. This indicates that the DEGs in alfalfa are primarily involved in immune defense responses (Figure 6 and Figure S5, Table S11).

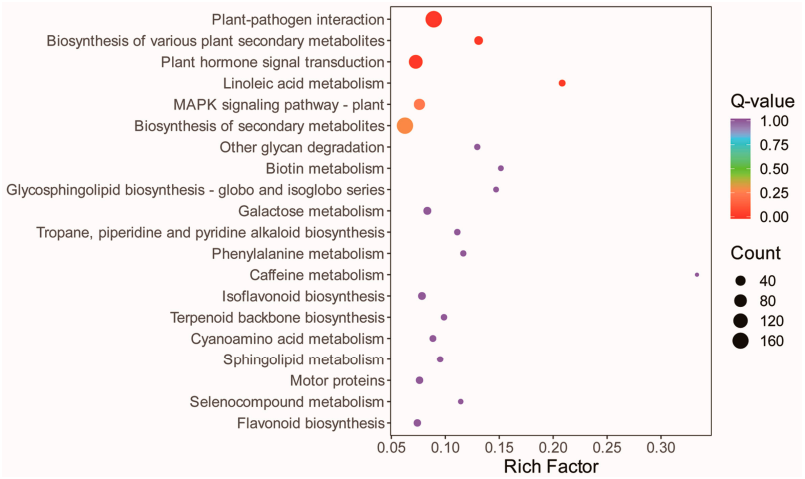


Figure 6. The KEGG enrichment analysis of alfalfa DEGs under straw composting. The horizontal axis represented the rich factor. The vertical axis represented the KEGG pathways. The larger the rich factor, the greater the enrichment level. The larger the dot, the more DEGs were enriched in the pathway. The redder the dot color, the more significant the enrichment was.

DEGs were also mapped using the KOG database, which contains eukaryotic orthologous groups. A total of 2178 DEGs were classified into 24 KOG categories, of which general function prediction only for the largest proportion (261, 24.32%), signal transduction mechanisms (122, 11.37%), "post-translational modifications, protein switching, concomitant proteins" (96, 8.95%), and posttranslational modification, protein turnover, chaperones (88, 8.2%) (Figure 7 and Table S12).

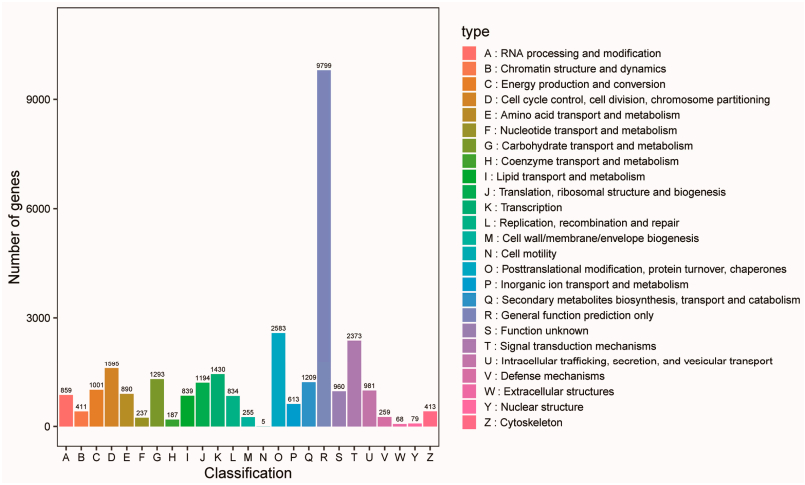


Figure 7. The KOG analysis of alfalfa DEGs under straw compost. The horizontal axis represented the functional classifications of KOG IDs, the vertical axis represented the number of included DEGs, and different classifications were represented by different colors. The legend contained codes with their functional description information.

3.6. RT-qPCR Verification for the Transcriptome Sequencing Results

Five important DEGs were selected with gene-specific primers for RT-qPCR analysis (Table S7). The gene expression trends were consistent with the transcriptome sequencing results (Figure 8).

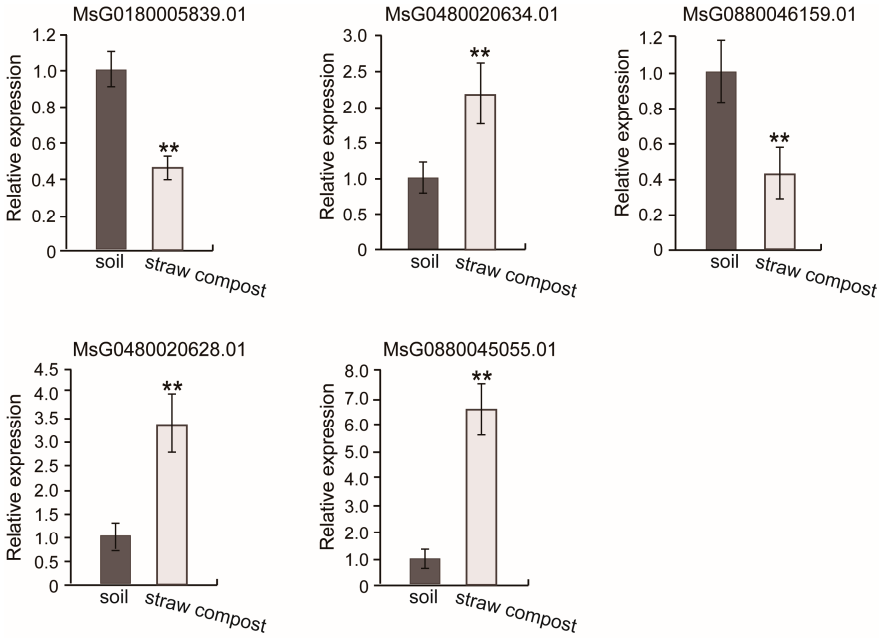


Figure 8. RT-qPCR analysis of the DEGs. The mRNA levels of five randomly selected DEGs were determined by RT-qPCR using the alfalfa 18S rRNA gene as an internal control. mRNAs from leaves grown in normal soil served as controls. Data are presented as the mean ± SE of three independent experiments. Asterisks denote statistically significant differences relative to normal soil: ** $p < 0.01$, as assessed by Student's *t*-test.

4. Discussion

4.1. Application of Straw Compost in Agricultural Production

Crop straws are rich in nutrients, and compost derived from their fermentation effectively improves the agricultural ecological environment while increasing crop yields. For example, the application of corn straw compost enhanced soil microbial activity and abundance, as well as carbon decomposition metabolism, thereby increasing cucumber yield [35]. Similarly, the use of straw compost improved the photosynthetic capacity and carbon metabolic enzyme activity of corn, significantly boosting its yield [13]. Rice straw compost, with its high nutrient content and strong enzyme activity, can optimize soil nutrient composition, promote rice growth, and enhance stress resistance in rice plants [36]. Straw compost can also be combined with other growth-promoting substances for synergistic effects. For instance, mixing rice straw compost with food residues and other organic wastes promoted lignin degradation, accelerated the succession of soil bacterial communities, improved compost fertility, and significantly increased Chinese cabbage yield [37]. Likewise, applying straw fertilizer fermented together with manure to wheat [38], rice [39] and Chinese cabbage [40] promoted seed germination and seedling growth, enhanced antioxidant capacity, and improved both quality and yield. The combination of straw fertilizer and microbial agents has also been shown to alleviate soil salinization and compaction, thereby increasing bell pepper yields [41]. Straw composting reduces the soil pH value and increases the soil organic matter content, thereby promoting the absorption of N, P and K of alfalfa. At the same time, it enhances the chlorophyll content in the leaves and the accumulation of crude protein, ultimately facilitating the growth of alfalfa in terms of plant height and biomass accumulation [42]. In addition, cultivation substrates made by composting straw with vermiculite and perlite significantly improved soil physicochemical properties, providing a well-aerated, moisture-retentive, and nutrient-rich environment that effectively promoted chrysanthemum growth [43]. In conclusion, the application of straw compost has been shown to increase both crop yield and quality, while also enabling

the resource utilization of agricultural waste. Its use is therefore of great significance for the sustainable development of agricultural production.

Northeast China is a major grain-producing region, generating a large amount of crop straw each year. However, because of the relatively low temperatures in spring, there are fewer fungal species suitable for low-temperature straw fermentation, and composting technology under such conditions remains limited. This makes large-scale outdoor straw composting difficult to implement, leading to significant waste of renewable straw resources. In our earlier work, we applied microbial agents adapted to straw decomposition in cold regions to ferment discarded crop straw mixed with Longmin black pig manure. When this compost was combined with natural soil and used for rice cultivation, we observed increases in plant height, yield, and stress resistance. In the present study, the same compost was mixed with natural soil for alfalfa cultivation, and its impact on alfalfa growth was examined (Figure 1). The results showed that compost application promoted plant height, root development, and leaf growth in alfalfa, confirming that it also has a growth-promoting effect on forage crops. These findings provide theoretical support for the industrial-scale promotion of low-temperature straw fermentation technology in cold regions, as well as for the broader application of straw composting in both agriculture and animal husbandry.

4.2. Low-Temperature Fermentation of Straw Compost Promotes the Growth of Alfalfa by Optimizing the Soil Microenvironment and the Physical and Chemical Properties of Alfalfa

Long-term agricultural production has led to nutrient depletion, pH imbalance, and even salinization problems, all of which severely restrict normal crop growth. Research indicates that alfalfa grows best in soils with a pH of 6.5–7.5 [44]. In this study, the application of straw compost increased the rhizosphere soil pH of alfalfa from 6.48 to 6.88, bringing it closer to the optimal range and thereby improving alfalfa growth and development (Figure 3B). Soil pH, as a key indicator, undergoes constant dynamic changes. The effect of straw compost on pH regulation is influenced by the inherent properties of the soil, and alterations in soil physicochemical characteristics may further affect soil structure and the nutrient absorption efficiency of alfalfa. However, the specific mechanism by which straw compost regulates soil pH remains unclear and requires further investigation. Soil enzymes act as biological catalysts in the material cycle and energy flow of soil ecosystems, and their activity is often used to assess soil fertility and health. For example, sucrase, a key enzyme in the carbon cycle, regulates sucrose metabolism, influencing organic matter transformation and the energy supply of microorganisms. Urease catalyzes the hydrolysis of urea, locally increases soil pH, and participates in the nitrogen cycle, thereby improving nitrogen utilization efficiency. Through such mechanisms, these enzymes contribute to crop growth by enhancing the soil microenvironment [45]. In this study, straw compost application significantly increased the activities of urease and sucrase in the rhizosphere soil of alfalfa, creating more favorable conditions for its growth (Figure 3C). In addition, nitrogen, phosphorus, and potassium-the primary nutrients required for plant growth-directly affect crop development [46]. The application of straw compost significantly increased the levels of hydrolyzable nitrogen, available phosphorus, and available potassium in the rhizosphere soil of alfalfa, improving the quality of forage, and promoting livestock production (Figure 3A). Soil microorganisms, as active components of the soil ecosystem, influence nutrient cycling through their activity and community structure, thereby affecting crop growth, yield, and quality. In this study, straw compost application increased both fungal and actinomycete populations in the soil. Notably, the number of fungi was nearly three times that of the control, while the number of actinomycetes was more than five times higher (Figure 3D). This suggests that straw compost promotes organic matter decomposition and nutrient release by regulating microbial abundance and reshaping soil

microbial community structure, thus optimizing the soil environment for the growth of alfalfa. In conclusion, straw compost prepared by low-temperature fermentation promotes alfalfa growth by regulating and improving the soil microenvironment.

Research shows that plants under adverse conditions trigger oxidative responses, producing harmful metabolites such as malondialdehyde. To counter this, plants synthesize antioxidant enzymes such as SOD, POD, and CAT. By catalyzing ROS-scavenging reactions, these enzymes convert peroxides into low-toxic or non-toxic metabolic products, thereby alleviating the cellular damage caused by malondialdehyde. The Al^{3+} dissolved in acidic soil will induce the production of ROS in the root systems of alfalfa seedlings and inhibit their growth. Alfalfa can regulate the expression of antioxidant enzyme genes and flavonoid synthesis genes through *MYB/WRKY* transcription factors to remove ROS and chelate Al^{3+} to cope with acid aluminum stress [47]. Thus, the activity of antioxidant enzymes can serve as an indicator of a plant's ability to decompose harmful substances. In this study, straw compost significantly increased the activity of SOD, POD, CAT, and related enzymes in alfalfa (Figure 2A), enhancing its adaptability to environmental stress. Results from DAB and NBT staining, as well as H_2O_2 content measurements, showed that straw compost reduced the accumulation of H_2O_2 and O^{2-} in alfalfa leaves (Figure 2B,C). These findings suggest that straw compost application induces higher antioxidant enzyme activity, thereby improving ROS removal efficiency. Chlorophyll, as a key photosynthetic pigment, plays an essential role in plant photosynthesis. This study also found that straw compost application significantly increased chlorophyll a, chlorophyll b, and carotenoid contents in alfalfa leaves (Figure 2D), thereby improving photosynthetic efficiency and promoting biomass accumulation. Taken together, these results indicate that straw compost promotes alfalfa growth by enhancing stress resistance and improving adaptation to adverse environments. To further test this inference, saline-alkali soil (pH 10.16) from the Andar region of Northeast China was used as the experimental substrate, with straw compost applied during alfalfa cultivation. The results showed that compost application significantly promoted alfalfa growth under saline-alkali stress (Figure 9), confirming that straw compost effectively enhances alfalfa's stress resistance.

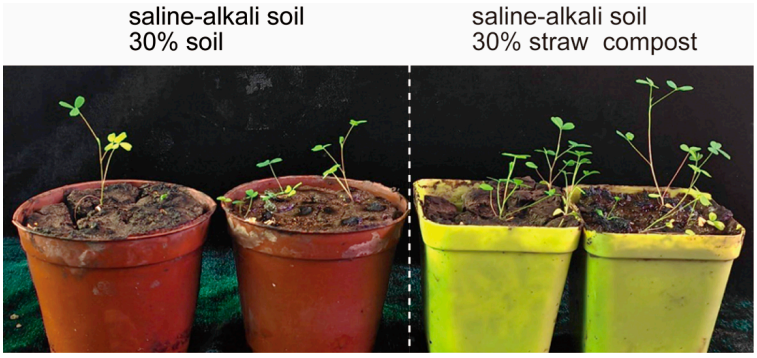


Figure 9. Effect of straw composting on alfalfa under saline alkali stress. The alfalfa seedlings grown in normal soil for 7 days were transferred to saline alkali soil containing 30% normal soil or to saline alkali soil containing 30% straw compost; after 15 days, alfalfa growth was observed and photographed.

At present, there is still a lack of systematic and in-depth analysis of the molecular mechanisms through which straw composting promotes plant growth. In this study, transcriptome sequencing was used to compare the gene expression profiles of alfalfa grown in normal soil and in soil treated with straw compost. In total, 2178 DEGs were identified (Figure 4). Transcriptome analysis revealed that the DEGs contained genes related to salicylic acid and hypoxia responses. Meanwhile, physiological index analysis

demonstrated that the application of straw compost promoted the activity of oxidation-related enzymes in alfalfa and increased the changes in chlorophyll content (Figure 4B and Table S9). GO functional annotation analysis revealed that these DEGs were significantly enriched in pathways central to crop growth and development, including responses to salicylic acid, responses to hypoxia, and responses to decreased oxygen levels (Figure 5). These biological processes are directly involved in regulating the physiological metabolism and stress adaptability of alfalfa and thus have a crucial impact on its overall growth and development. KEGG pathway enrichment analysis further showed that the DEGs were significantly enriched in plant hormone signal transduction, general metabolic pathways, biosynthesis of secondary metabolites, and plant–pathogen interactions (Figure 6). These results suggest that straw composting primarily influences hormone regulation, stress pre-adaptation, biological control, and metabolic support mechanisms, which in turn promote alfalfa growth and enhance biomass accumulation.

Therefore, we have found that the low-temperature–fermented straw compost used in this study is a fertilizer that promotes the growth of various crops. It optimizes the soil microenvironment required for alfalfa growth by regulating rhizosphere soil pH, increasing soil nutrient content, enhancing soil enzyme activity, and boosting the abundance of beneficial microorganisms. In addition, straw composting enhanced the activity of stress response-related enzymes in alfalfa, reduced ROS content, increased leaf photosynthesis, and regulated the expression of key genes involved in physiological processes such as hormone regulation and stress response. Together, these effects promoted the material cycle and energy flow in alfalfa, achieving the dual goals of soil optimization and improved yield (Figure 10). Looking ahead, we plan to integrate metabolomics and microbiomics to analyze the molecular networks through which straw composting regulates alfalfa growth and to cultivate alfalfa mutants with significant DEGs. Such efforts will help to clarify the functional and mechanistic impacts of straw composting on alfalfa.

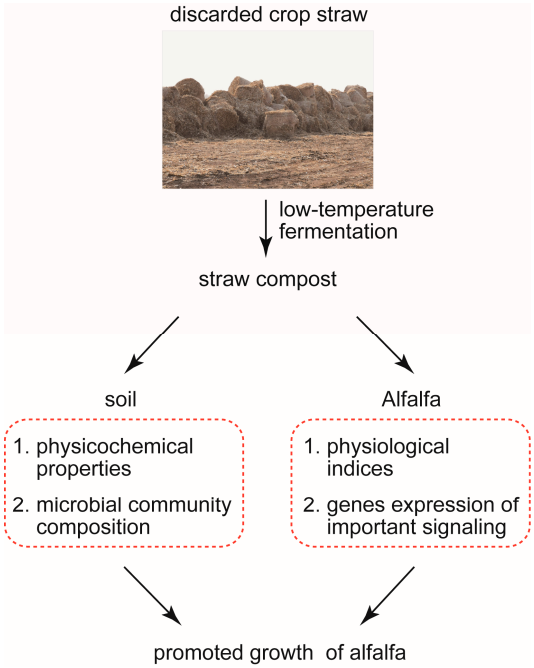


Figure 10. The possible mode of low-temperature fermentation straw composting promoted the growth and yield of alfalfa. Low-temperature-fermented straw compost promoted alfalfa growth by modulating soil physicochemical properties and microbial abundance, as well as by altering gene expression related to physiological traits and key signaling pathways in alfalfa.

5. Conclusions

In this study, the addition of 10%, 30% 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 alfalfa leaf chlorophyll content and enzyme activities related to stress responses, which positively affected alfalfa stress resistance. In addition, the application of straw compost influenced the expression of genes in alfalfa 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 alfalfa, and affected the expression of critical signaling pathway genes, thereby promoting alfalfa growth. However, the current research only focuses on alfalfa, and the roles of other crops are still unclear, and there is a lack of protein or metabolic level verification. In the subsequent research, the applicability and effect differences in straw composting on major crops such as wheat and corn will be verified, and the metabolic changes in alfalfa under straw composting treatment will be analyzed to reveal the key metabolic pathways.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy15122723/s1, Figure S1: Collection of straw compost; Figure S2: Effect of straw composting on alfalfa growth; 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 alfalfa; Table S1: Comparative analysis of blade length of alfalfa; Table S2: Effects of straw compost on various physiological indices of alfalfa; Table S3: Effects of straw compost on alfalfa leaf quality; Table S4: Soil physicochemical properties under straw compost; Table S5: The effect of straw compost on enzyme activity of soil; Table S6: The effect of straw compost on soil microbial abundance; Table S7: List of primers used for real-time PCR in this study; Table S8: The number of samples used in the experiment; Table S9: List of DEGs identified from RNA-Seq analysis; Table S10: GO enrichment list of differential genes under straw fertilizer treatment conditions; Table S11: KEGG enrichment list of differential genes under straw fertilizer treatment conditions; Table S12: KOG annotation list of differentially expressed genes under straw fertilizer treatment conditions.

Author Contributions: J.W. and Z.L. conceived and designed the experiments, and J.W., Z.Z., Y.L. and Z.L. wrote the manuscript. Z.Z., W.S., L.C. and H.C. performed the experiments. All authors have read and agreed to the published version of the manuscript.

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