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Effects of Low-Temperature Stress During the Grain-Filling Stage on Carbon–Nitrogen Metabolism and Grain Yield Formation in Rice

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Abstract: Interactions between carbon and nitrogen metabolism are essential for balancing source–sink dynamics in plants. Frequent cold stress disrupts these metabolic processes in rice and reduces grain yield. Two rice cultivars (DN428: cold-tolerant; SJ10: cold-sensitive) were subjected to 19 °C low-temperature stress at full-heading for varying lengths of time to analyze the effects on leaf and grain metabolism. The objective was to track carbon–nitrogen flow and identify factors affecting grain yield. Low-temperature stress significantly reduced the activity of nitrate reductase (NR), glutamine synthetase (GS), glutamate synthase (GOGAT), glutamate dehydrogenase (GDH), glutamic oxaloacetic transaminase (GOT), and glutamic pyruvic transaminase (GPT), in functional leaves compared to the control. This reduction decreased nitrogen accumulation, inhibited chlorophyll synthesis, and slowed photosynthesis. To preserve intracellular osmotic balance and lessen the effects of low temperatures, sucrose, fructose, and total soluble sugar levels, as well as sucrose synthase (SS) and sucrose phosphate synthase (SPS) activities, surged in response to low-temperature stress. However, low-temperature stress significantly reduced the activity of adenosine diphosphate glucose pyrophosphorylase (AGPase), granule-bound starch synthase (GBSS), soluble starch synthase (SSS), and starch branching enzyme (SBE). At the same time, low-temperature stress reduced the area of vascular bundles and phloem, making it difficult to transport carbon and nitrogen metabolites to grains on time. The response of grains to low-temperature stress differs from that of leaves, with prolonged low-temperature exposure causing a gradual decrease in carbon and nitrogen metabolism-related enzyme activities and product accumulation within the grains. The insufficient synthesis of starch precursors and carbon skeletons results in significantly lower thousand-grain weight and seed-setting rates, ultimately contributing to grain yield loss. This decline was more pronounced in inferior grains compared to superior grains. Compared to SJ10, DN428 exhibited higher values across various indicators and smaller declines under low-temperature stress, suggesting enhanced cold-tolerance and a greater capacity to maintain grain yield stability.

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1. Introduction

Rice (*Oryza sativa* L.), as with wheat, maize, and sugarcane, is a major food crop that contributes significantly to global food security, accounting for half of the world's crop production [1]. Rice yield depends on maintaining the perfect temperature [2]. However, rising global temperatures are causing more regular, intense, and extended low-temperature stress, resulting in an annual decline of 300–5000 million tons of rice production [3,4]. Heilongjiang Province, a major grain-producing region in China with high latitude and high altitude, is especially prone to these impacts, with severe cold damage that leads to yield reductions of more than 20% [5,6]. Therefore, there is an urgent need to explore the mechanism of cold-tolerance in rice, to maintain rice yields under low-temperature stress [7].

Rice may be subjected to low-temperature stress for varied amounts of time and intensity during different growth phases, impacting its growth and development to varying degrees [8]. The grain-filling period is a determinant stage for rice yield formation [9]. Prior to this stage, carbon and nitrogen assimilates produced by source organs, primarily leaves, are mainly stored in vegetative organs. During the heading and grain-filling stages, grains become the predominant carbon and nitrogen sink, deriving their required carbon and nitrogen primarily from leaf photosynthesis and nitrogen remobilization [10]. Studies have shown that the optimal temperature range for rice grain-filling is 22–27 °C [11]. Within this temperature range, leaves utilize complex photosynthetic biochemical mechanisms to capture light energy for the reduction and assimilation of CO_2 and NO_3^-, forming carbohydrates and amino acids, respectively, which are then efficiently transported to grains through phloem loading, vascular bundle transport, and phloem unloading processes [12,13]. During this period, the carbon–nitrogen metabolism between source and sink maintains a dynamic balance, conducive to enhancing the grain-filling rate and promoting grain-filling and yield improvement [14]. However, the carbon–nitrogen assimilation ratio is dynamic. Under low-temperature stress, leaves, as temperature-sensitive organs, exhibit multiple physiological disorders, including inhibited photosynthetic pigment synthesis, reduced stomatal conductance, and decreased CO_2 assimilation capacity [15,16]. These changes restrict ATP generation from photosynthesis and respiration, leading to competition between carbon and nitrogen metabolism. The activities of key carbon metabolism-related enzymes, such as adenosine diphosphate glucose pyrophosphorylase (AGPase), soluble starch synthase (SSS), granule-bound starch synthase (GBSS), and starch branching enzyme (SBE), are significantly reduced, leading to limited photosynthetic assimilate synthesis [17]. Under these conditions, limited photosynthates are preferentially allocated to maintain basic physiological functions rather than nitrate reduction [18]. Meanwhile, reduced activities of nitrogen metabolism-related enzymes, such as nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS), and glutamate synthase (GOGAT), further inhibit amino acid and protein synthesis, affecting rice growth, development, and stress resistance [19–24]. Additionally, low temperatures induce structural changes in the phloem, further impeding assimilate transport to grains. Grain position differences lead to significant variations in filling capacity. Superior grains located on the top primary branches and inferior grains on secondary branches show distinct differences in assimilate allocation [25]. When source organ supply capacity is limited, assimilates are preferentially allocated to superior grain, leading to further disparities in filling rate and plumpness between superior and inferior sinks, ultimately affecting rice yield and quality.

While carbon and nitrogen metabolism under low-temperature stress has been extensively studied in rice, the dynamics between source–sink relationships and superior–inferior grain development remain underexplored, particularly regarding their synergistic responses to cold stress. This comparative study selected two contrasting japonica rice cultivars from Heilongjiang Province, DN428 (cold-tolerant) and SJ10 (cold-sensitive), by exposing them to controlled low-temperature stress (19 °C) during the critical full-heading stage, with progressive treatment durations (1–7 days). We hypothesized that differential cold-tolerance between cultivars would manifest through distinct patterns of carbon–nitrogen allocation, with DN428 maintaining better source–sink coordination to sustain grain development under cold stress. This systematic investigation not only elucidates the physiological basis of cold-tolerance divergence between cultivars but also provides novel insights into source–sink regulation strategies for improving rice productivity under low-temperature conditions.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

This study was conducted from April to early September 2022 and 2023 at the experimental site of the Northeast Agricultural University, in Harbin City, Heilongjiang Province, China (longitude: 126°22′–126°50′ E; latitude: 45°34′–45°46′ N). Two rice varieties with similar growth periods but significant differences in cold-tolerance, DN428 (a cold-tolerant variety) and SJ10 (a cold-sensitive variety), were selected as experimental materials [26]. DN428 and SJ10 have approximately 138 and 137 days of fertility, respectively, from emergence to maturity, and both varieties have approximately 40 days from the grain-filling stage to harvest. Both of them are suitable for planting in the second-season temperate zone of Heilongjiang Province.

2.2. Experimental Design

Rice seeds were sown on 21 April 2022 and 24 April 2023 and transplanted on 26 May 2022 and 27 May 2023.

2.2.1. Field Experiment

The experiment used a split-plot design. Rice variety was chosen as the subplot factor, with each subplot measuring 18 m^2. Row length was 6 m, number of rows was 10, the distance between rows was 30 cm, the distance between hills was 10 cm, the planting density was 3 plants per hill, and there were 3 repetitions. The plots were separated using a large soil ridge. Mixed fertilizer (total nutrients ≥48%) was applied using general field production practices, with a 6:3:1 ratio of base to panicle fertilizer, and spike fertilizer was applied at the stage of young spike differentiation (1 July 2022 and 2 July 2023). During the full-heading stage (approximately 80% of the rice plants had headed on 27 July 2022 and 28 July 2023), we used low root water temperature irrigation for 1, 3, 5, and 7 days, labeled as C1, C3, C5, and C7, respectively, with the water temperature set to 19 °C. The water layer was maintained at a depth of roughly 20 cm. Deep ground water (8–9 °C) and pool water exposed to sunlight (22–24 °C) were combined to create the cold water [27,28]. The water temperature was set at 19 °C, close to the temperature at which cold damage occurs during the grain-filling of rice in Heilongjiang Province [29]. An automatic water quality monitoring device was used to monitor regularly. The control treatment (C0) used pool water exposed to sunlight placed alongside other treatments. Based on the National Bureau of Statistics data, the average temperature during the grain-filling stage in Heilongjiang

Province from 2012 to 2021 was 23.09 °C. However, during the experimental period (2022 and 2023), the average temperature during the grain-filling stage was 1.45% lower than the ten-year average, respectively. The average air temperature in the field during the low-temperature treatment period was 21.63 °C (2022) and 21.46 °C (2023) (Figure 1). A supplemental file (Figure S1A,B) contains the experimental design's field map.

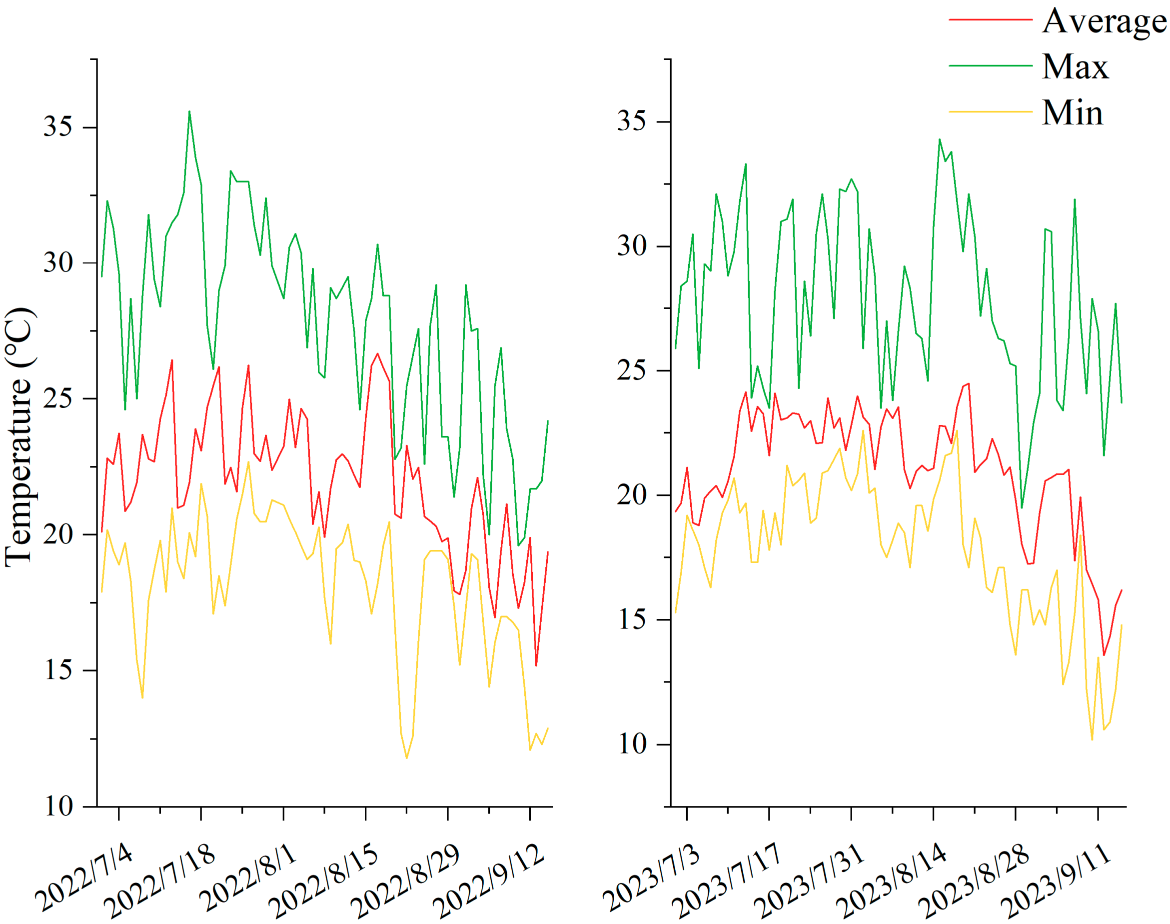

Figure 1. The temperature trends from the grain-filling stage to maturity in 2022 and 2023.

2.2.2. Pot Experiment

This experiment was conducted in pots simultaneously in 2022. The design of the pot trial was as follows: The experiment was conducted in a random arrangement [30–32]. Leakproof plastic pots (30 cm × 28 cm) were selected, and each pot was filled with 10 kg of sieved and mixed black soil. The planting density was set at 4 hills per pot, with 3 plants per hill, resulting in 210 pots per variety and 420 pots total. At the full-heading stage, uniformly growing plants were selected and marked. For each variety, 168 pots were transferred to a low-temperature artificial climate chamber set at 19 °C (close to the low-temperature stress threshold during the rice grain-filling stage) for 1, 3, 5, and 7d, labeled as C1, C3, C5, and C7, respectively, with 14 pots per treatment and 3 replicates. Each variety had 42 pots in a room-temperature artificial climate chamber set to 25 °C and was labeled as C0 treatment (control). After the low-temperature treatment, these pots were immediately moved to the room-temperature artificial climate chamber. Apart from the temperature settings, all other conditions in the artificial climate chambers were the same, with light intensity at 30,000 lx, photoperiod from 5:00 to 19:00, carbon dioxide concentration at 400 ppm, and relative humidity at 75%.

2.3. Field Sampling and Lab Analyses

2.3.1. Photosynthetic Characteristics

Low-water-temperature treatment started at the full-heading stage. Sampling began on the second day after all treatments' completion and was collected every 5 days until grain maturity, totaling 7 times. From 9:00 to 11:00, for each treatment, 3 hills were selected and the SPAD value of the main stem sword leaf was determined using a SPAD-502 chlorophyll meter (Minolta Camera Co. Ltd., Osaka, Japan) with 3 replicates. Net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO_2 concentration (Ci), and transpiration rate (Tr) of the main stem sword leaf were measured using a CIRAS-3 portable photosynthetic system (PP Systems, Haverhill, MA, USA), also with 3 replicates.

2.3.2. Sugar Content of Leaves and Grains

Sampling Method

Sampling was carried out in the morning of the same day that photosynthesis was measured. For each treatment, 3 hills with uniform growth were selected for 3 replicates. Fully expanded functional leaves and corresponding panicles were collected. Grains from the panicles were sorted into superior and inferior grains, taking the top 3 primary branches for superior grains and the bottom 3 secondary branches for inferior grains, removing empty grains.

Measurement Methods

The samples were dried at 105 °C for 30 min and then dried to a constant weight at 80 °C in an oven. They were ground and sieved through a 100-mesh sieve, then stored in a desiccator for the measurement of non-structural carbohydrate content.

Total soluble sugars were determined using the anthrone colorimetric method, sucrose and fructose were measured by the phenol colorimetric method [33], and starch was analyzed using the dual-wavelength method [34].

2.3.3. Activity of Starch and Sucrose Metabolism Enzymes in Leaves and Grains

Sampling Method

Sampling was carried out in the morning of the same day that photosynthesis was measured. For each treatment, 3 hills with uniform growth were selected for 3 replicates. Fully expanded functional leaves and corresponding panicles were collected. Grains from the panicles were sorted into superior and inferior grains, taking the top 3 primary branches for superior grains and the bottom 3 secondary branches for inferior grains, removing empty grains. The samples were treated with liquid nitrogen and stored in a −80 °C freezer for later use.

Measurement Methods

The activities of sucrose synthase (SS) and sucrose phosphate synthase (SPS) were measured according to Nakamura [35]. The activities of starch branching enzyme (SBE), adenosine diphosphate glucose pyrophosphorylase (AGPase), soluble starch synthase (SSS), and granule-bound starch synthase (GBSS) were determined using appropriate kits purchased from Beijing Box Biotech Co., Ltd., Beijing, China; the names of the kits are ADP-Glucose Pyrophosphorylase (AGP) Activity Assay Kite, Soluble Starch Synthase (SSS) Activity Assay Kit, Granule-Bound (GBSS) Starch Synthase Activity Assay Kit, and Starch Branching Enzyme (SBE) Activity Assay Kit.

2.3.4. Nitrogen Accumulation of Leaves and Grains

Sampling Method

Sampling was carried out in the morning of the same day that photosynthesis was measured. For each treatment, 3 hills with uniform growth were selected for 3 replicates. Fully expanded functional leaves and corresponding panicles were collected. Grains from the panicles were sorted into superior and inferior grains, taking the top 3 primary branches for superior grains and the bottom 3 secondary branches for inferior grains, removing empty grains. The samples were dried at 105 °C for 30 min and then dried to a constant weight at 80 °C in an oven. They were ground and sieved through a 100-mesh sieve, then stored in a desiccator for the measurement of non-structural carbohydrate content.

Measurement Methods

The nitrogen content in various organs was determined using an automated plant component analyzer (Skalar Primacs SN100-2C, Skalar Corp., Norcross, Georgia) through the combustion method.

2.3.5. Activity of Nitrogen Metabolism Enzymes in Leaves and Grains

Sampling Method

Sampling was carried out in the morning of the same day that photosynthesis was measured. For each treatment, 3 hills with uniform growth were selected for 3 replicates. Fully expanded functional leaves and corresponding panicles were collected. Grains from the panicles were sorted into superior and inferior grains, taking the top 3 primary branches for superior grains and the bottom 3 secondary branches for inferior grains, removing empty grains. The samples were treated with liquid nitrogen and stored in a −80 °C freezer for later use.

Measurement Methods

The activity of nitrate reductase (NR) was measured via the sulfonamide colorimetry method [36]. The activity of glutamine synthetase (GS) was based on the ferric chloride colorimetric method [37]. The activity of glutamate synthase (GOGAT) was conducted according to Singh and Srivastava [38]. The determination of glutamate dehydrogenase (GDH) activity was conducted according to González-Moro [39]. The activities of glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) were measured by Liang [40].

2.3.6. Histomorphological Observation

The areas of vascular bundles and phloem in the functional leaves and the panicle nodes s were observed according to paraffin sections. Sampling was carried out on the day after the end of all low-water-temperature treatments, 10 functional leaves and panicle nodes of the main stems were selected for each treatment and replicated three times, and about 1 cm of leaves was cut in the middle of the functional leaves; the middle part of the panicle node was intercepted with a length of about 0.3 cm, which was kept in FAA fixative and changed every 12 h, and was then rinsed, dehydrated, made transparent, dipped in wax, embedded, sliced, spread and baked, dewaxed, stained, and finally placed under a microscope (Nikon Eclipse 80i, Nikon Corporation, Tokyo, Japan) to take pictures [41], and the vascular bundle area and phloem area were determined by Image J 2.0.

2.3.7. Grain Yield and Rice Quality

During the mature stage, 9 hills growing rice plants were selected for each treatment, with 3 repetitions, to measure panicle traits, grain yield, and yield components. Grain-filling dynamics were calculated according to the work of Zhu [42]. Rice grain quality analysis was conducted according to Qiu [43].

2.4. Formulae

The data collected from the experiment were processed using normalization and then visualized as a heatmap. The normalization formula is defined as follows [44]:

$$x\prime = \frac{x - {}_{\min}(x)}{{}_{\max}(x) - {}_{\min}(x)} \tag{1}$$

To introduce the temperature accumulation for each sampling period, this paper calculates the Growing Degree Days (GDDs, °C·d) for each sampling period, calculated with reference to Wypych and Liu [45,46] and adjusted appropriately, as follows:

$$GDD = \sum_{da}^{db} GDi \tag{2}$$

$$GDi = \left\{ \begin{array}{l} 0, T_{avg} < T_j \\ T_{avg} - T_j, T_{avg} \geq T_j \end{array} \right\} \tag{3}$$

Among them, *da* and *db* represent the dates of each sampling day and the day before the next sampling; T_{avg} is the daily average temperature, T_j is the minimum growth temperature for rice (T_j = 10 °C), and *GDi* is the cumulative value of the daily average temperature exceeding the minimum growth temperature on the *i*-th day.

To evaluate the extent of low-temperature stress experienced by rice during cold treatment, this study calculated the Cold Degree Days (CDDs, °C·d) for each treatment, calculated according to the work of Liu [47] and adjusted appropriately, as follows:

$$CDD = \sum_{dm}^{dn} CDi \tag{4}$$

$$CDi = \left\{ \begin{array}{l} 0, T_{min} > T_h \\ T_{min} - T_h, T_{min} \leq T_h \end{array} \right\} \tag{5}$$

Among them, *dm* and *dn* represent the dates when the low-temperature treatment starts and ends, respectively; T_{min} is the daily minimum temperature, T_h is the temperature close to the low-temperature damage threshold for rice in Heilongjiang Province (T_h = 19 °C) [29], and *CDi* is the cumulative value of the daily minimum temperature below the damage threshold on the *i*-th day.

2.5. Statistical Analysis

We used the SPSS 27.0 software package for data statistics and analysis. Analysis of variance (ANOVA) was used to evaluate the effects of low-temperature stress on nitrogen and carbon metabolism as well as grain yield-related indicators. Due to the high correlation between the data, ridge regression analysis was used to determine the causal relationships between variables. Ridge regression analysis is considered an improved form of multiple linear regression analysis, which introduces a regularization term (also known as a penalty term) to address the issue of high correlation among variables [48]. Since the data obtained from the pot experiment and the field experiment show a constant change trend, the variance analysis found that most of the data showed no significant difference (Tables S1 and S10). The field data are more meaningful in actual production and presented as the data obtained from the field test. As the trends in the data obtained for 2022 between the indicators are similar to those for 2023, the data are presented for 2022 except for grain yield and its components. The ridge regression analyses of grain yield and its components use the average values for 2022 and 2023. Graphs were created using Origin 2024 [49].

3. Results

3.1. Effects of Low-Temperature Stress During Grain-Filling Stage on Rice Carbon Metabolism

3.1.1. Photosynthesis

According to the variance analysis of the field experiment and pot experiment, only Pn has a significant difference ($p < 0.05$) between them (Table S1). The following data are presented based on the results of field experiments.

This study found that there are significant or highly significant differences in the physiological parameters of rice functional leaves (Table S2), including net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), intercellular CO_2 concentration (Ci), SPAD, sucrose content (Suc), and nitrogen accumulation (N), among various rice varieties, treatments, and their interactions. Under specific conditions, certain physiological indices show no significance after full-heading: Pn at 18 and 33 d, Gs at 23 d, Ci at 8, 23, and 33 to 38 d, and SPAD value at 13 d in different varieties; Pn at 18 to 23 and 38 d, Tr at 23 to 33 d, Gs at 8 to 18 and 33 to 38 d, Ci at 8 and 23 to 38 d, SPAD at 38 d, Suc at 13 d, and N at 8 to 23 and 33d between varieties and treatments.

Photosynthesis-related indicators (Tr, Gs, Ci, and N) in rice functional leaves decline gradually after full-heading (Figure 2), with low-temperature stress exacerbating this decline, which intensifies with prolonged stress. The most significant reduction occurs under the C7 treatment, with varying timing of peak reductions among indicators. For DN428 and SJ10, the maximum Tr reduction occurs at 28 and 38 days post-heading (43.87% and 57.45%, respectively), N at 28 and 33 days (37.36% and 53.19%), Gs at 33 days (69.28% and 74.05%), and Ci at 38 days (31.43% and 36.36%). Overall, DN428 consistently demonstrates higher values and smaller reductions across all indicators as opposed to SJ10.

Pn and SPAD levels change with different low-temperature treatments (Figure 2). For DN428 under C0 to C3 treatment and SJ10 under C0 and C1 treatment, both Pn and SPAD decreased after full-heading. In contrast, DN428 under C5 and C7 treatment and SJ10 under C3 to C7 treatment showed an initial increase followed by a decrease, peaking 13 days after full-heading. Compared to C0 treatment, prolonged low-temperature stress (3–7 days) significantly reduced Pn and SPAD, with reductions becoming sharper over time, reaching their lowest at 38 days after full-heading under C7 treatment. The decreases for DN428 were 39.94% for Pn and 29.84% for SPAD, while for SJ10, they were 46.21% and 39.91%, respectively.

Suc in functional rice leaves follows a single-peak pattern after full-heading (Figure 2), with peaks at 23 d for DN428 and 28d for SJ10. Suc significantly increased under low-temperature stress at 8d after full-heading for most treatments (except C1 treatment, which was not significant) with the highest increase under C7 treatment (13.81% and 27.47%). Between 13 and 38 d after full-heading, Suc significantly increased under all low-temperature treatments for DN428 and under C1 and C3 treatment for SJ10 (except for DN428 under C1 treatment and SJ10 under C1 treatment from 33 to 38d, which were not significant). Suc under C5 and C7 treatment for SJ10 were significantly lower than under C0 treatment.

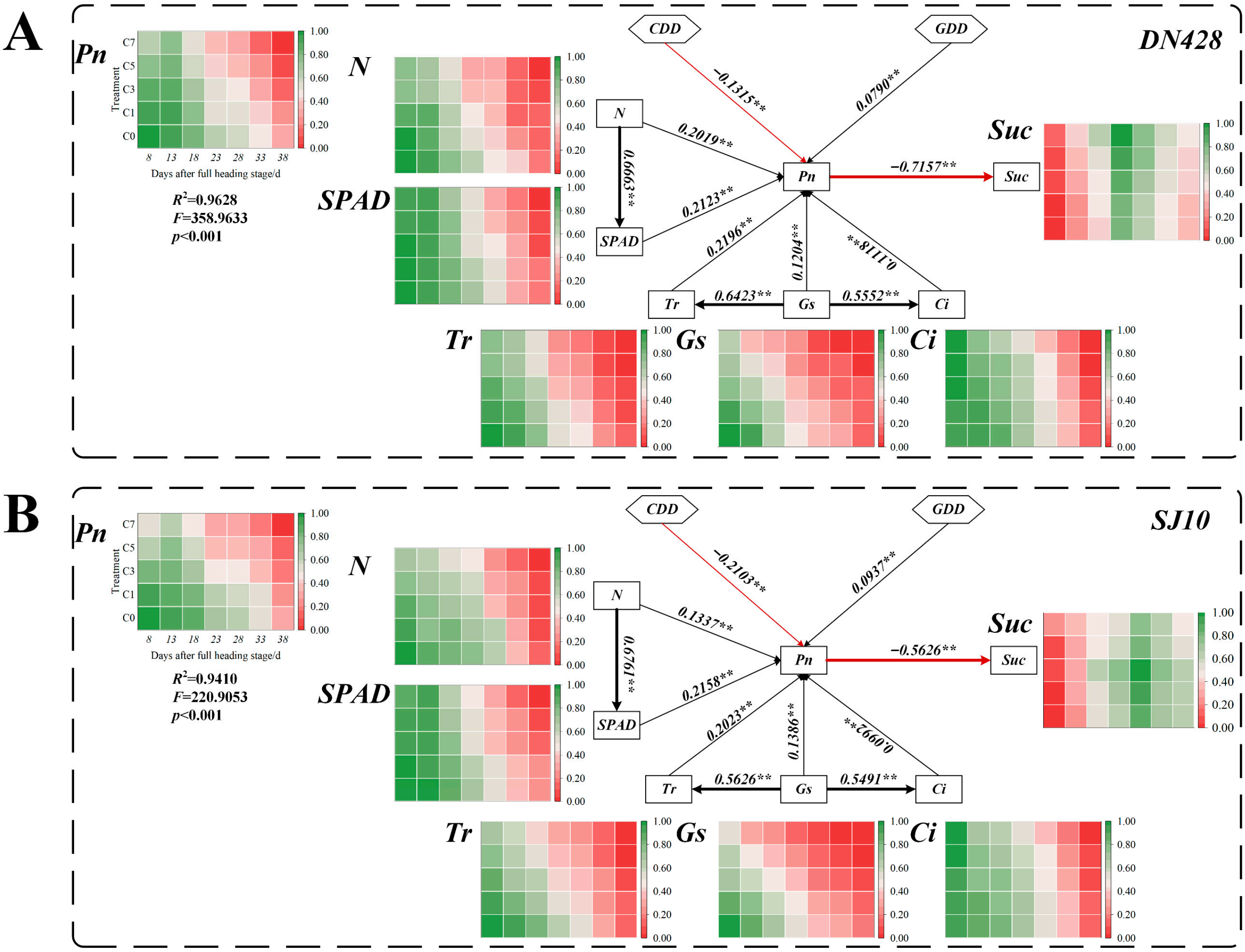

Figure 2. Trends in photosynthesis-related indices in functional leaves of rice under low-temperature stress during the grain-filling stage and results of ridge regression analysis. (**A**) DN428. (**B**) SJ10. Suc represents the sucrose content in functional leaves of rice. N represents the nitrogen accumulation in the functional leaves of rice. CDD represents Cold Degree Day. GDD represents Growing Degree Day. The values are the standardized coefficients, i.e., path coefficients, calculated through ridge regression analysis. ** indicates significance at $p < 0.01$. The black lines represent a positive correlation. The red lines represent a negative correlation. The bold lines represent path coefficients greater than 50%.

Figure 2 shows that the coefficients of determination (R^2_{Pn}) for functional leaves of DN428 and SJ10 under low-temperature stress during the grain-filling stage were 0.9628 and 0.9410, respectively, both with p-values under 0.001, indicating a strong model fit. The direct effects of Tr, Gs, Ci, SPAD, and N on Pn were positive and significant for both varieties. For DN428, the path coefficients ranked Tr > SPAD > N > Gs > Ci, while for SJ10, they were SPAD > Tr > Gs > N > Ci. The cumulative direct effects of the top three indicators on Pn were 0.6338 for DN428 and 0.5567 for SJ10. Gs significantly positively affected Tr and Ci, while N positively influenced SPAD. DN428 had higher path coefficients than SJ10, all over 0.5, indicating that Gs and N could indirectly impact Pn through Tr, Ci, and SPAD, despite their small direct effects. Pn also had a significant negative direct effect on Suc, with coefficients of −0.7157 for DN428 and −0.5626 for SJ10.

Under low-temperature stress during the grain-filling stage, CDD significantly negatively affected Pn in DN428 and SJ10, with path coefficients of −0.1315 and −0.2103, respectively. GDD considerably positively influenced Pn, with coefficients of 0.0790 for

DN428 and 0.0937 for SJ10. DN428 showed stronger resistance to low-temperature stress than SJ10. The key traits affecting Pn differed between cultivars: Tr was crucial for DN428, while SPAD was more important for SJ10. In DN428, low temperatures led to stomatal closure and reduced transpiration, lowering photosynthesis. SJ10 had weaker photosynthetic capacity, limited by pigment concentration. Low SPAD in SJ10 kept Pn suppressed even with high Tr. Additionally, Suc negatively regulated photosynthesis, worsening the inhibition of photosynthetic activity.

3.1.2. Sugar Metabolism

Total Soluble Sugar Content and Sucrose Metabolism-Related Enzyme Activity in Functional Leaves of Rice

According to Table S3, significant or highly significant differences are observed in the contents of sucrose (Suc), fructose (Fru), and total soluble sugar (TSS), as well as the activities of sucrose synthase (SS) and sucrose phosphate synthase (SPS) in the functional leaves of varieties, treatments, and their interactions. Under specific conditions, certain physiological indices show no significance after full-heading: Fru at 18 and 33 d, TSS at 28 to 38 d in varieties, TSS at 33 d in treatments, Suc at 13 d, Fru and SS at 38 d, and SPS at 8 d between varieties and treatments.

Figure 3 shows that as the growth period advances, the contents of Suc, Fru, and TSS and the activities of SS and SPS in rice functional leaves follow a single-peak curve that increases initially and then decreases after full-heading. For DN428, all indicators peak 23 d after full-heading, while SJ10 peaks 5 days later than DN428. DN428's peak values under all low-temperature stress treatments and SJ10 C1 and C3 treatments are significantly higher than the C0 treatment (except for DN428 C1 treatment, which is not significant). SJ10 C5 and C7 treatments are considerably lower than the C0 treatment. The maximum peak growth is observed in the C7 treatment for DN428 and C3 treatment for SJ10 (DN428: 10.30%, 7.46%, 8.38%, 5.35%, 9.89%; SJ10: 7.27%, 5.05%, 3.00%, 2.96%, 6.13%).

Compared to C0 treatment (Figure 3), 8 d after full-heading, Suc and TSS significantly increased in all low-temperature treatments (except the C1 treatment, which is not significant), with the C7 treatment showing the largest increase. Subsequently, there were differences between the two varieties across all low-temperature treatments for DN428 and C1 and C3 treatments for SJ10, showing significant increases in Suc and TSS (except for the DN428 C1 treatment and SJ10 C1 treatment at 33–38 d, which were not significant), while the SJ10 C5 and C7 treatments were significantly lower than the C0 treatment.

Compared to the C0 treatment (Figure 3), under low-temperature stress (1–7 days), Fru content and SS and SPS activity significantly increase before reaching their peak values (except for DN428 C1 treatment and SJ10 C5 and C7 treatments 18–28 d after full-heading, which show decreased peak values). A total of 8 d after full-heading, the C7 treatment shows the largest increase in Fru, SS, and SPS (DN428: 35.65%, 23.14%, 21.34%; SJ10: 47.68%, 31.29%, 26.85%). However, after reaching peak values, all indicators decrease significantly. As the duration of low-temperature treatment increases, the rate of decrease for each indicator gradually increases, meaning the rate of decline is higher than in the C0 treatment.

Figure 3 shows that the coefficients of determination (R^2_{Suc}) for functional leaves of DN428 and SJ10 under low-temperature stress during the grain-filling stage were 0.9706 and 0.9278, respectively, both with p-values under 0.001, indicating a strong model fit.

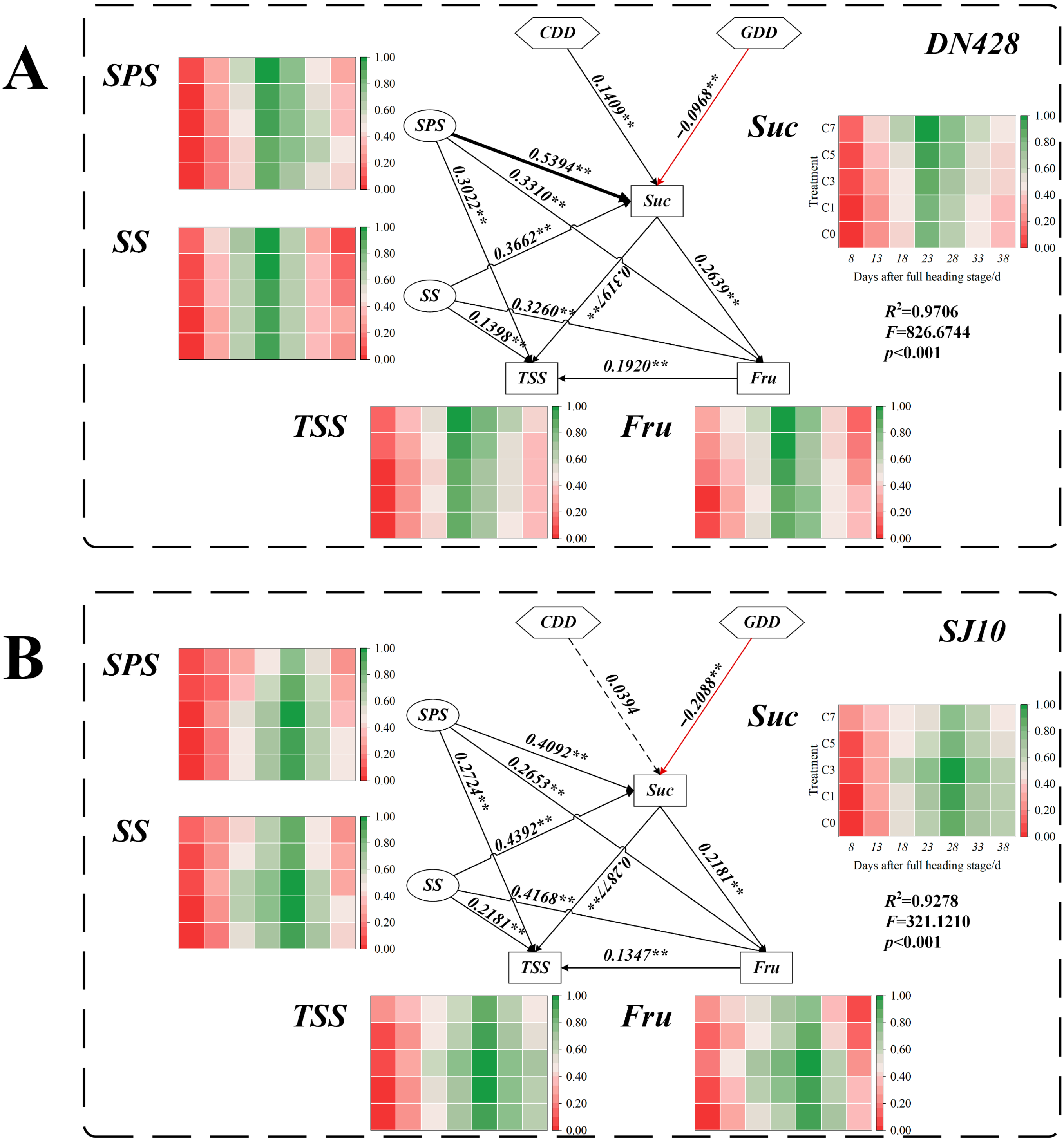

Figure 3. Trends in total soluble sugar content and sucrose metabolism-related enzyme activity in functional leaves of rice under low-temperature stress during the grain-filling stage and results of ridge regression analysis. (**A**) DN428. (**B**) SJ10. CDD represents Cold Degree Day. GDD represents Growing Degree Day. Suc represents the sucrose content in functional leaves of rice. Fru represents the fructose content in functional leaves of rice. TSS represents the total soluble sugar content in functional leaves of rice. SS represents the sucrose synthase activities in functional leaves of rice. SPS represents the sucrose phosphate synthase activities in functional leaves of rice. The values are the standardized coefficients, i.e., path coefficients, calculated through ridge regression analysis. ** indicates significance at $p < 0.01$. NS indicates not significant. The black lines represent a positive correlation. The red lines represent a negative correlation. The dashed lines indicate a non-significant correlation. The bold lines represent path coefficients greater than 50%.

The direct effects of SPS and SS on Suc, Fru, and TSS in functional leaves of DN428 and SJ10 are all positive and significant. The influence of SPS and SS activities on Suc varies between the two rice varieties; DN428 exhibits a higher SPS effect than SS, with path coefficients of 0.5394 and 0.3662, respectively, while SJ10 shows the opposite, with path coefficients of 0.4092 and 0.4392. The research results show that the activities of SPS and SS have a significant positive correlation with Fru and TSS. Different cold-resistant rice varieties exhibit differences in sugar metabolism regulation mechanisms. For the cold-sensitive rice variety SJ10, SS activity plays a major role in the accumulation of Fru and TSS, with the direct impact coefficient of SJ10's SS activity on Fru content being 21.79% higher than that of DN428, and the direct impact coefficient on TSS content increasing by 35.90%. In contrast, for the cold-resistant rice variety DN428, the direct impact coefficient of SPS activity on Fru content is 31.82% higher than that of SJ10, and the direct impact coefficient on TSS increases by 10.94%.

Under low-temperature stress during the grain-filling stage, GDD has a direct negative effect on Suc content in the functional leaves of DN428 and SJ10, with path coefficients of 0.0968 and 0.2088, respectively. CDD positively influences Suc, but the effect on SJ10 is insignificant, with path coefficients of 0.1409 and 0.0394. This indicates that low-temperature stress promotes the accumulation of Suc in functional leaves by stimulating the activities of SS and SPS, which helps maintain cellular osmotic pressure and enhances the resistance of rice to low-temperature stress. Prolonged low-temperature stress (3–7 d) significantly reduces the content of TSS in the functional leaves of SJ10, and the TSS content under various low-temperature treatments in SJ10 is lower than that in DN428, indicating that SJ10 has less capability for Suc synthesis and decomposition under low-temperature stress and is more significantly affected by cold conditions.

Sugar Content and the Activity of Enzymes Related to Starch Metabolism in Superior and Inferior Grains of Rice

Analysis of variance (ANOVA) results (Table S4) indicate that the contents of Suc, Fru, and TSS in the superior and inferior grains of rice exhibit significant or highly significant differences among different varieties, treatments, and their interactions. Under specific conditions, certain physiological indices show no significance after full-heading: TSS at 23 d in superior grains among varieties, Suc at 18 and 28 d, Fru at 28 to 33 d in inferior grains among varieties, Suc at 33 d, Fru at 23 to 38 d in superior grains between varieties and treatments, and Fru at 28 to 38 d in inferior grains between varieties and treatments.

The analysis of variance (ANOVA) results (Table S5) show that the activities of adenosine diphosphate glucose pyrophosphorylase (AGPase), granule-bound starch synthase (GBSS), soluble starch synthase (SSS), and starch branching enzyme (SBE), as well as the contents of amylose, amylopectin, and amylum in superior grains of rice, exhibited significant or highly significant differences across different varieties, treatments, and their interactions. Under specific conditions, certain physiological indices show no significance after full-heading: GBSS at 28 and 38 d, SSS at 8 and 38 d, amylum at 33 d in varieties, GBSS at 8 to 23 d, SSS and SBE at 8 to 13 and 38 d, amylose at 8 to 13 and 33 d, amylopectin at 33 to 38 d, and amylum at 8 to 13 and 33 to 38 d between varieties and treatments.

The analysis of variance (ANOVA) results (Table S6) indicate that the activities of adenosine diphosphate glucose pyrophosphorylase (AGPase), granule-bound starch synthase (GBSS), soluble starch synthase (SSS), and starch branching enzyme (SBE), as well as the contents of amylose, amylopectin, and amylum in inferior grains of different rice varieties, showed significant or highly significant differences across different varieties, treatments, and their interactions. Under specific conditions, certain physiological indices show no significance after full-heading: AGPase at 28 d, GBSS at 8 to 23 and 33 to 38 d, amylose at 33 d in varieties, AGPase at 8 d, GBSS at 8 to 28 d, SSS at 8 and 33 d, SBE at 8 and 38 d, and amylose and amylum at 28 to 33 d between varieties and treatments.

As the growth period advances, the C0 treatment leads to a declining trend in the content of Suc, Fru, and TSS in the superior grains of rice after full-heading (Figure 4). The low-temperature treatments for the DN428 and the C1 and C3 treatments for the SJ10 in superior grains follow the same trend as the C0 treatment. However, the C5 and C7 treatments for the SJ10 superior grains, as well as the low-temperature treatments for the inferior grains of both varieties, exhibit an initial increase followed by a decrease in these sugar contents, and prolonged low-temperature treatment (5–7 d) delays time to peak.

Compared to the C0 treatment, at 8 d after full-heading, the Suc and TSS in grains under various low-temperature treatments are significantly lower than those of the C0 treatment (except C1 treatment for DN428), with the C7 treatment showing the greatest reduction. For the superior grains, the decrease in Suc for DN428 and SJ10 is 5.59% and 8.64%, respectively, and for TSS it is 12.94% and 42.79%, respectively. For the inferior grains, the decreases are 41.64% and 44.92% for Suc and 42.82% and 52.40% for TSS. At 38 d after full-heading, the Suc and TSS contents in the grains under various low-temperature treatments are significantly higher than those of the C0 treatment (the C1 treatment was non-significant), with the C7 treatment showing the greatest increase.

In comparison to the C0 treatment, the Fru in the superior grains under various low-temperature treatments is significantly lower at 8 d and 28 to 38 d after full-heading (except for DN428 C1 and SJ10 from 33 to 38 d of C1). For the inferior grains, the Fru is significantly lower at 8 to 13 d and 33 to 38 d (except for C1 from 33 to 38 d), which showed further decrease with prolonged low-temperature treatments. At 38 d, the C7 treatment shows the largest decrease in Fru, with reductions of 49.78% for DN428 superior grains and 65.12% for inferior grains, and for SJ10 it is 62.09% and 74.92%, respectively.

The content of amylose, amylopectin, and amylum in the superior and inferior grains of DN428 and SJ10 shows an increasing trend after full-heading. Compared with the C0 treatment, the content of amylose in the superior and inferior grains under low-temperature treatment significantly decreases from 8 to 23 d (except for the inferior grains on the 8th day), with the decrease gradually increasing as the number of treatment days increases. From 28 to 38 d, the amylose content in the superior and inferior grains under C3, C5, and C7 treatments significantly increases (except for DN428 superior grains at 33 d). The largest decrease in amylose content occurs at 13 d for C7 treatment in superior grains, with reductions of 46.76% for DN428 and 52.33% for SJ10; the largest decrease in inferior grains occurs at 18 d, with reductions of 38.67% for DN428 and 43.95% for SJ10. The largest increase in amylose content occurs at 28 d for C7 treatment in both superior and inferior grains, with increases of 7.20% and 6.63% for DN428 and 8.54% and 11.11% for SJ10, respectively.

The activities of AGPase, GBSS, SSS and SBE in the grains show a tendency to increase initially and then decrease as the growth period advances after full-heading, forming a single-peak curve. Compared to C0 treatment, the peak activities of AGPase, SSS, and SBE in the superior and inferior grains under different low-temperature treatments decrease significantly, and this decrease gradually increases with the number of days of treatment. The time taken for the peak activity to be reached is also delayed.

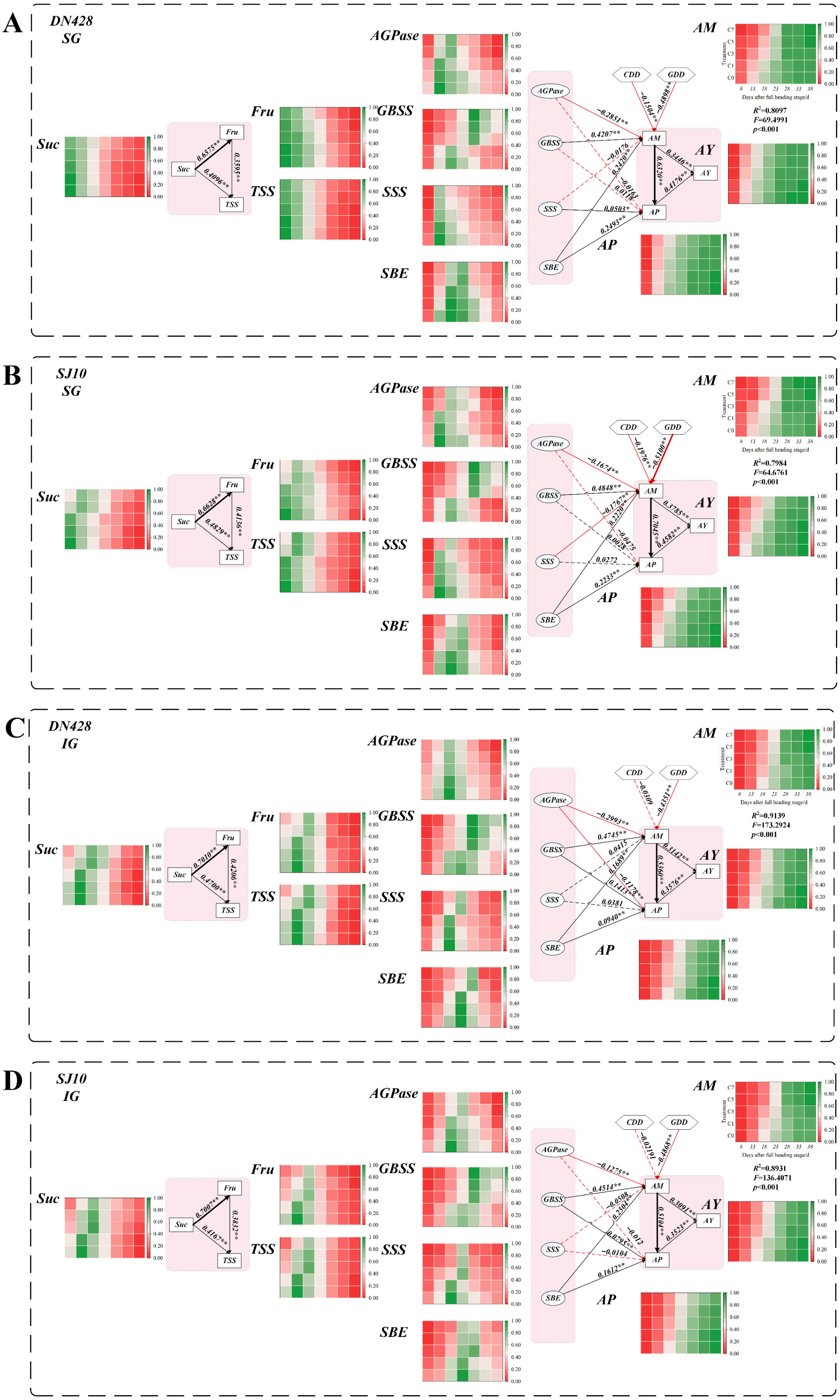

Figure 4. Trends in sugar content and starch metabolism-related enzyme activity in grains of rice under low-temperature stress during the grain-filling stage and results of ridge regression analysis.

(**A**) DN428 superior grains. (**B**) SJ10 superior grains. (**C**) DN428 inferior grains. (**D**) SJ10 inferior grains. CDD represents Cold Degree Day. GDD represents Growing Degree Day. Suc represents the sucrose content in grains of rice. Fru represents the fructose content in grains of rice. TSS represents the total soluble sugar content in grains of rice. AM represents amylose content in grains of rice. AP represents amylopectin content in grains of rice. AY represents amylum content in grains of rice. AGPase represents the adenosine diphosphate glucose pyrophosphorylase activities in inferior grains of rice. GBSS represents the granule-bound starch synthase activities in inferior grains of rice. SSS represents the soluble starch synthase activities in grains of rice. SBE represents the starch branching enzyme activities in grains of rice. The values are the standardized coefficients, i.e., path coefficients, calculated through ridge regression analysis. The symbol * indicates significance at $p < 0.05$. ** indicates significance at $p < 0.01$. NS indicates not significant. The black lines represent a positive correlation. The red lines represent a negative correlation. The dashed lines indicate a non-significant correlation. The bold lines represent path coefficients greater than 50%.

The AGPase activity in the superior grains under the C0, C1, and C3 treatments reaches its highest activity at 13 d after full-heading, while the C5 and C7 treatments are delayed by 5 days compared to the former three. For the inferior grains, all treatments of DN428 and the SJ10 C0, C1, and C3 treatments reach the highest activity at 18 d, while the SJ10 C5 and C7 treatments are also delayed by 5 days compared to the former ones. The GBSS activity both in the superior and inferior grains reaches its highest at 18 and 23 d, respectively, under the C0 and C1 treatments after full-heading; although the C3, C5, and C7 treatments are delayed by 5 days, their peaks are significantly higher than the former ones. The SSS activity in the superior grains under all treatments reaches its highest at 13 d after full-heading, except for the C7 treatment, which is delayed by 5 days. For the inferior grains, C0 and C1 treatments and C3 treatment for DN428 peak at 18 d, whilst C5 and C7 treatments and C3 treatment for SJ10 are delayed by 5 days. The SBE activity both in superior and inferior grains of DN428 reaches its highest at 13 d under the C0, C1, and C3 treatments, and C5 and C7 treatments are delayed by 5 days. For SJ10, the SBE activity both in superior and inferior grains reaches its highest at 23 d under the C0 and C1 treatments and the C3, C5, and C7 treatments are delayed by 5 days.

Figure 4 shows that the coefficients of determination (R^2_{AM}) for the DN428 and SJ10 superior grain starch synthesis model are 0.8097 and 0.7984, respectively. The coefficient of determination for the inferior grains of both varieties is 12.87% and 11.86% higher than that of the superior grains, respectively. The p-values for all four models are less than 0.001, indicating that all four models are statistically significant, and the fit of the models for inferior grains is better than that for superior grains.

The effect of Suc on Fru in the superior and inferior grains of DN428 and SJ10 is positive and significant and the path coefficients for the inferior grains are 6.61% and 5.41% higher than those for the superior grains, respectively. The activity of AGPase has a significant negative correlation with amylose content. The path coefficients for DN428’s superior and inferior grains are 0.2851 and 0.2993, respectively, and SJ10 is 41.28% and 57.40% lower than DN428, respectively. The activity of GBSS has a significant positive correlation with amylose content. The path coefficients for DN428’s superior and inferior grains are 0.4207 and 0.4745, respectively, and for SJ10, the path coefficient is 15.24% higher than DN428 in superior grains and 5.12% lower than DN428, respectively. Both CDD and GDD show negative effects on amylose accumulation but the effect of CDD on starch accumulation in inferior grains was not significant

3.2. Effects of Low-Temperature Stress During Grain-Filling Stage on Rice Nitrogen Metabolism

3.2.1. Functional Leaves

The analysis of variance (ANOVA) results (Table S7) show that the nitrogen accumulation (N) in functional leaves of different rice varieties, along with the activities of nitrate reductase (NR), glutamine synthetase (GS), glutamate synthase (GOGAT), glutamate dehydrogenase (GDH), glutamic oxaloacetic transaminase (GOT), and glutamic pyruvic transaminase (GPT), exhibited significant or highly significant differences among varieties, treatments, and their interactions. Significant or highly significant differences were observed for N, GOGAT, and GDH under variety–treatment interactions. Under specific conditions, certain physiological indices show no significance after full-heading: GOT and GPT at 8 d in varieties, NR and GOT at 38 d, GS at 28 to 38 d, GPT at 33 to 38 d in treatments, and N at 8 to 13 and 33 d between varieties and treatments.

As shown in Figure 5, with an advancement in the growth process, N in functional leaves of rice, as well as the activities of NR, GS, GOGAT, GDH, GOT, and GPT, shows a decreasing trend. Compared with the C0 treatment, low-temperature stress significantly reduced N in functional leaves of rice, as well as the activities of NR, GS, GOGAT, GOT, and GPT from 8 to 23 d after full-heading. The reduction is more pronounced with the extension of low-temperature stress, with the greatest reduction observed in the C7 treatment on the 8 d after full-heading (N observed at 28 d). The reduction in the DN428 indices was 26.73%, 55.61%, 82.52%, 53.79%, 29.22%, and 20.27%, respectively, while the reduction in the SJ10 indices was 43.17%, 74.33%, 127.27%, 99.00%, 65.09%, and 113.63%, respectively. The activity of GDH was significantly higher than that of the C0 treatment from 8 to 28 d, and the increase was more pronounced with the extension of low-temperature stress, with the greatest increase observed in the C7 treatment in the 13 d (DN428: 30.01%, SJ10: 61.71%). N, NR, GS, GOGAT, GOT, and GPT activities in the functional leaves of SJ10 were all significantly lower than those of DN428.

From Figure 5, it can be seen that under low-temperature stress during the grain-filling and seed-setting stage, the coefficients of determination (R^2_N) for the nitrogen metabolism model of functional leaves in DN428 and SJ10 are 0.9567 and 0.9163, respectively, and both *p*-values are less than 0.001, indicating that the models have a good fit and are statistically significant.

The direct effects of NR, GS, GOGAT, GOT, and GPT activities in the functional leaves of DN428 and SJ10 on the nitrogen accumulation in functional leaves are all positive and significant (the effects of GOT activity on nitrogen accumulation in DN428 and GPT activity on nitrogen accumulation in SJ10 are not significant). The path coefficients, from largest to smallest, are GDH > GOGAT > GS > GPT > NR > GOT, and GDH > NR > GOGAT > GS > GOT > GPT. The cumulative path coefficients for the first three indicators' direct effects on N are 0.6202 and 0.6545, respectively. Under low-temperature stress during the grain-filling stage, GDD has a direct positive effect on N in DN428 and SJ10, with the path coefficient in SJ10 being 108.23% higher than that in DN428. CDD has a direct negative effect on N in DN428 and SJ10, with the path coefficient in SJ10 being 79.02% higher than that in DN428.

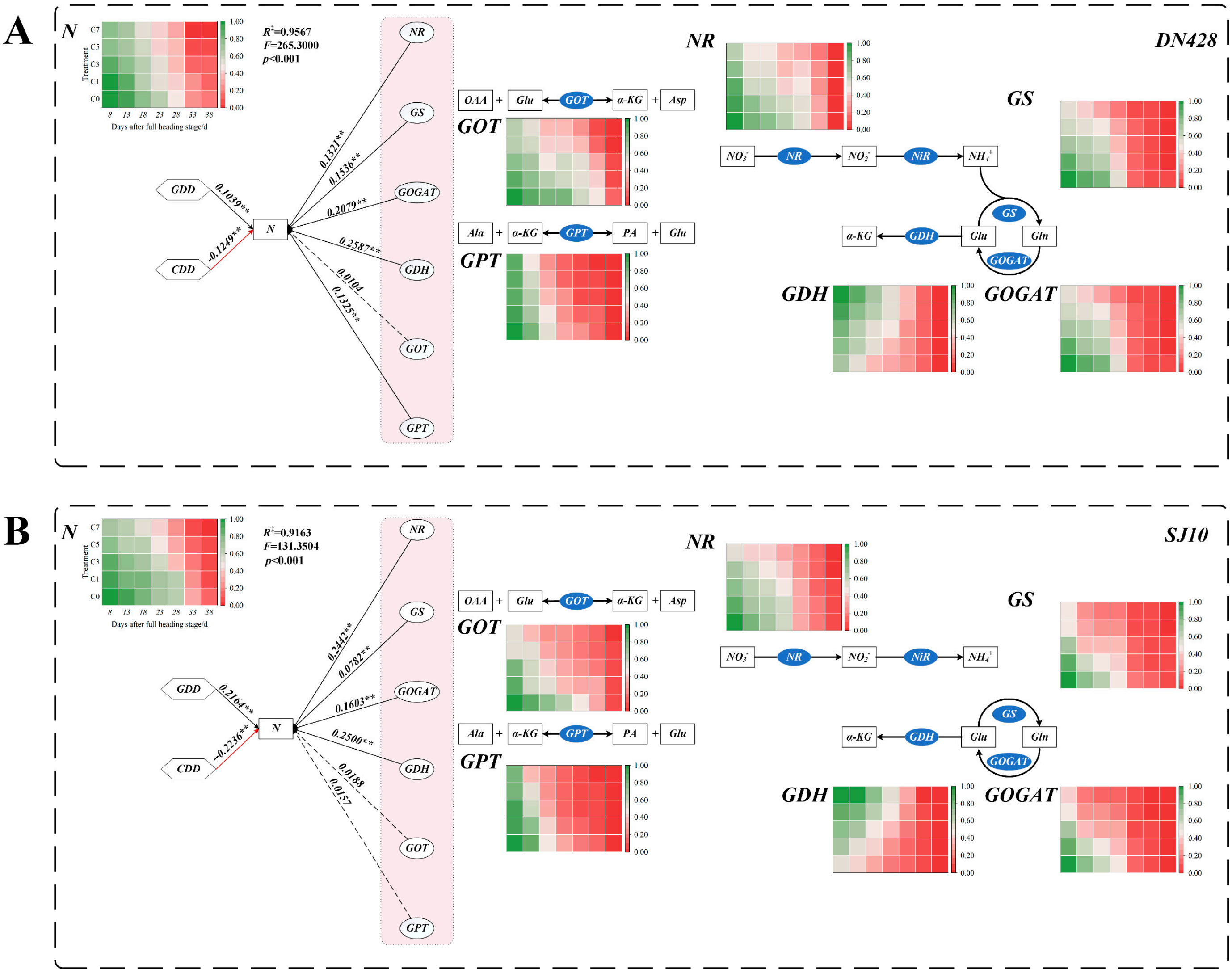

Figure 5. Trends in nitrogen accumulation and nitrogen metabolism-related enzyme activity in functional leaves of rice under low-temperature stress during the grain-filling stage and results of ridge regression analysis. (**A**) DN428. (**B**) SJ10. CDD represents Cold Degree Day. GDD represents Growing Degree Day. N represents the nitrogen accumulation in the functional leaves of rice. NR represents the nitrate reductase activities in functional leaves of rice. GS represents the glutamine synthetase activities in functional leaves of rice. GOGAT represents the glutamate synthase activities in functional leaves of rice. GDH represents the glutamate dehydrogenase activities in functional leaves of rice. GOT represents the glutamic oxaloacetic transaminase activities in functional leaves of rice. GPT represents the glutamic pyruvic transaminase activities in functional leaves of rice. ** indicates significance at $p < 0.01$. NS indicates not significant. The black lines represent a positive correlation. The red lines represent a negative correlation. The dashed lines indicate a non-significant correlation.

3.2.2. Superior and Inferior Grains

The analysis of variance (Table S8) shows that the N and protein content in superior grains, as well as the activities of GS, GOGAT, GDH, and GPT, exhibit significant or highly significant differences among varieties, treatments, and their interactions. Under specific conditions, certain physiological indices show no significance after full-heading: GS at 18 to 33 d, GOGAT at 18 to 28 d, GPT at 8 to 13 d, protein at 18 and 33 d in varieties, GS and GOGAT at 28 to 38 d, GDH at 33 to 38 d, GPT at 8 and 38 d in treatments, N at 13, 23, and 33 to 38 d, GS at 8 to 38, GOGAT at 13, 28, and 38, and GDH and protein at 28 to 38 d between varieties and treatments.

Analysis of variance (Table S9) indicates significant or highly significant differences in N and protein content in inferior grains, as well as the activities of GS, GOGAT, GDH, GOT, and GPT, under different varieties, treatments, and their interactions. Under specific conditions, certain physiological indices show no significance after full-heading: GPT at 8 d in varieties, GS at 33 to 38 d, GOT and GPT at 38 d in treatments, N at 8 and 33 d, GS at 8 and 28 to 38 d, GOGAT 33 to 38 d, GOT at 8 to 38 d, GPT at 8, 18 and 38 d, and protein at 8 to 13 d between varieties and treatments.

From Figure 6, it can be observed that compared with C0 treatment, the activities of GS, GOGAT, GOT, and GPT in both superior and inferior grains of rice under low-temperature stress during the grain-filling stage significantly decreased from 8 to 23 d after full-heading, and the decrease became more pronounced with the extension of low-temperature stress, with no significant change in enzyme activity at 38 days after heading. In contrast, the activity of GDH in both superior and inferior grains of rice under low-temperature stress significantly increased from 8 to 23 d, with the increase becoming more pronounced with the extension of low-temperature stress, and no significant change in enzyme activity at 33 d.

The low-temperature stress during the grain-filling stage had a significant impact on the GS, GOGAT, GDH, GOT, and GPT in the inferior grains, while the decrease in enzyme activity in the superior grains was significantly lower than in the inferior grains. There were significant differences between varieties under low-temperature stress during the grain-filling and seed-setting stage, with the nitrogen metabolic enzyme activity in both superior and inferior grains of SJ10 being significantly lower than in DN428.

The accumulation of N in the rice leaves significantly decreased after heading, while N accumulation in both superior and inferior grains significantly increased after full-heading, reaching the highest at maturity. Under low-temperature stress during the grain-filling stage, N in the superior grains was significantly higher than in the inferior grains, and both the nitrogen concentration and accumulation in the superior and inferior grains of SJ10 were significantly lower than in DN428, with a greater change range in N of SJ10 than of DN428.

From Figure 6, it can be seen that under low-temperature stress during the grain-filling and seed-setting stage, the coefficient of determination (R^2_N) for the nitrogen metabolism model of superior and inferior grains in both DN428 and SJ10 was greater than 0.9 and the *p*-values were all less than 0.001, indicating that the models had good fit and were statistically significant.

The direct effects of GS, GOGAT, GOT, and GPT activities in superior and inferior grains of DN428 and SJ10 on the nitrogen accumulation in superior and inferior grains were all negative effects (except for the positive and significant effect of GS activity in SJ10 superior grains on grain nitrogen accumulation). Under low-temperature stress, the activity of nitrogen metabolism enzymes in the grains decreases, but nitrogen accumulation does not stop. This may be due to the restriction of nitrogen accumulation rate, thereby limiting nitrogen accumulation. GDD and CDD showed significant negative effects on the nitrogen accumulation in superior and inferior grains of DN428 and SJ10, that is, low-temperature stress during the grain-filling and seed-setting stage reduced the activity of related enzymes. Since the nitrogen in the grains mainly comes from the leaves, this is reflected as a negative effect on the path coefficients.

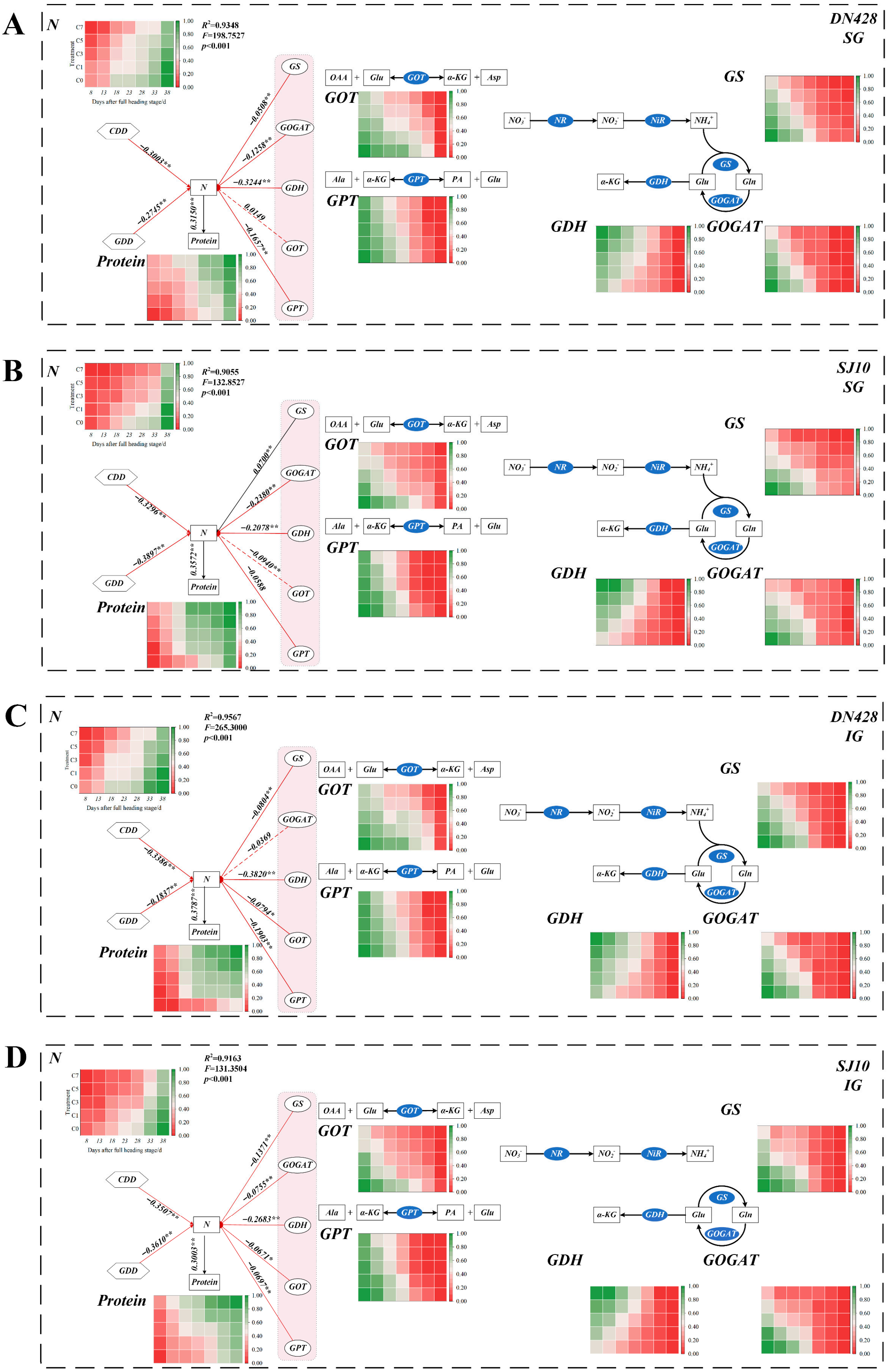

Figure 6. Trends in nitrogen accumulation and nitrogen metabolism-related enzyme activity in the grains of rice under low-temperature stress during the grain-filling stage and results of ridge regression analysis. (**A**) DN428 superior grains. (**B**) SJ10 superior grains. (**C**) DN428 inferior grains.

(**D**) SJ10 inferior grains. CDD represents Cold Degree Day. GDD represents Growing Degree Day. N represents the nitrogen accumulation in the grains of rice. GS represents the glutamine synthetase activities in the grains of rice. GOGAT represents the glutamate synthase activities in the grains of rice. GDH represents the glutamate dehydrogenase activities in the grains of rice. GOT represents the glutamic oxaloacetic transaminase activities in the grains of rice. GPT represents the glutamic pyruvic transaminase activities in the grains of rice. The values are the standardized coefficients, i.e., path coefficients, calculated through ridge regression analysis. The symbol * indicates significance at $p < 0.05$. ** indicates significance at $p < 0.01$. NS indicates not significant. The black lines represent a positive correlation. The red lines represent a negative correlation. The dashed lines indicate a non-significant correlation.

3.3. Effects of Low-Temperature Stress During the Grain-Filling Stage on the Formation and Accumulation of Rice Yield

3.3.1. The Grain-Filling Dynamics

According to Table 1, under low-temperature stress during the grain-filling stage, the final weight of a kernel (A), the maximum grain-filling rate (GRmax), and the mean grain-filling rate (GRmean) of both superior and inferior grains of rice significantly decreased (except for the C1 treatment of DN428 superior grains). This decrease became more pronounced as the duration of treatment increased, reaching its maximum in the C7 treatment. Compared to the C0 treatment, the C7 treatment of DN428 showed decreases of 9.71%, 32.76%, and 30.38% in superior grains and 25.57%, 46.75%, and 48.00% in inferior grains for these indicators, respectively. For SJ10, the decreases in superior grains were 18.26%, 50.96%, and 56.72%, while in inferior grains they were 34.77%, 73.02%, and 69.05%.

Table 1. Effects of low-temperature stress during the grain-filling stage on the grain-filling dynamics of superior and inferior grains in rice.

Variety	**Treatment**	**Grain Position**	**Equation Parameters**				**Grouting Parameters**			
			A	**B**	**K**	**N**	**Tmax (d)**	**GRmax**	**GRmean**	**D (d)**
DN428	C0	SG	25.08	13.99	0.23	0.84	12.07	1.54	1.03	24.40
		IG	22.15	71.44	0.23	1.41	17.11	1.13	0.74	29.75
	C1	SG	24.81	27.49	0.25	1.12	12.71	1.51	1.00	24.73
		IG	21.49	47.05	0.21	1.21	17.60	1.05	0.70	30.91
	C3	SG	24.57	4.34	0.19	0.46	11.91	1.40	0.95	25.98
		IG	20.56	24.94	0.18	0.91	18.72	0.93	0.62	32.97
	C5	SG	23.90	1.27	0.16	0.19	11.77	1.29	0.87	27.40
		IG	18.99	130.06	0.21	1.51	21.63	0.85	0.56	34.10
	C7	SG	22.86	1.99	0.16	0.26	12.92	1.16	0.79	28.99
		IG	17.64	153.25	0.20	1.45	23.62	0.77	0.50	35.00
SJ10	C0	SG	25.06	16.77	0.24	0.87	12.30	1.57	1.05	23.87
		IG	21.51	117.70	0.24	1.64	17.72	1.09	0.71	30.22
	C1	SG	23.92	13.49	0.22	0.81	12.76	1.40	0.94	25.57
		IG	19.52	48.25	0.20	1.22	18.36	0.92	0.61	32.13
	C3	SG	23.19	7.18	0.19	0.57	13.29	1.27	0.86	27.02
		IG	18.64	186.76	0.21	1.72	21.82	0.82	0.54	34.67
	C5	SG	21.88	5718.53	0.35	3.39	21.17	1.13	0.71	30.71
		IG	17.13	69.10	0.18	1.20	22.24	0.74	0.49	35.13
	C7	SG	21.19	1783.77	0.28	2.52	23.12	1.04	0.67	31.86
		IG	15.96	29.12	0.15	0.79	24.50	0.63	0.42	37.89

SG represents superior grains. IG represents inferior grains. A represents the final weight of a kernel. B represents the initial parameter. K represents the growth rate parameter. N represents the shape parameter. GRmax represents the maximum grain-filling rate. Tmax represents the time reaching the maximum grain-filling rate. GRmean represents the mean grain-filling rate. D represents the active grain-filling stage.

Regarding the time reaching the maximum grain-filling rate (Tmax) and the active grain-filling stage (D), both DN428 and SJ10 showed increases in Tmax and D for superior and inferior grains as the low-temperature treatment duration extended (the difference in Tmax for DN428 superior grains under various low-temperature treatments was not significant compared to the control). The largest increases were observed in the C7 treatment. Compared to the C0 treatment, the C7 treatment resulted in a 38.05% increase in Tmax for DN428 inferior grains, while SJ10 showed increases of 87.97% and 38.26% in Tmax for superior and inferior grains, respectively. The increases in D for DN428 were 18.81% and 17.65% for superior and inferior grains, while for SJ10, they were 33.47% and 25.38%.

In conclusion, the grain-filling dynamics of rice grains showed significant differences between varieties and grain positions. Under low-temperature stress, superior grains consistently outperform inferior grains in all indicators and show smaller decreases. Moreover, the cold-tolerant variety DN428 performed in all indicators compared to the cold-sensitive variety SJ10, with smaller decreases under low-temperature stress. This indicated that DN428 demonstrates stronger resistance to low-temperature conditions, with grains showing a greater ability to absorb and convert photosynthetic products, better maintenance of physiological activities during grain-filling, and more efficient utilization of non-structural carbohydrates produced by photosynthesis, thereby enhancing its yield potential.

3.3.2. Panicle Traits

As shown in Table 2, the panicle traits of rice in 2022 and 2023 showed extremely significant differences among varieties. Panicle length, primary branch number, and secondary branch number did not show significant differences among treatments; however, the number of empty grains in primary and secondary branches and spikelet fertility exhibited extremely significant differences among treatments. Additionally, the number of empty grains in primary branches showed significant differences between years and between year × variety interactions, while the number of empty grains in secondary branches exhibited extremely significant differences among varieties × treatments.

Table 2. Effects of low-temperature stress during the grain-filling stage on the rice panicle traits.

Year	Variety	Treatment	Panicle Length	Primary Branch Number	Empty Primary Branch Grains	Secondary Branch Number	Empty Secondary Branch Grains	Spikelet Fertility (%)
2022	DN428	C0	18.51 a	10.10 a	4.27 d	16.87 a	6.56 e	97.14 a
		C1	18.01 a	9.63 a	4.49 d	16.33 a	7.49 d	95.29 b
		C3	18.29 a	10.00 a	5.01 c	16.73 a	9.41 c	90.66 c
		C5	17.90 a	9.87 a	5.61 b	16.00 a	11.04 b	87.42 d
		C7	17.93 a	9.70 a	5.92 a	15.80 a	13.12 a	83.64 e
	SJ10	C0	15.91 a	9.63 a	5.03 c	14.31 a	8.13 e	94.34 a
		C1	15.69 a	9.47 a	5.46 c	13.97 a	10.52 d	91.73 b
		C3	15.69 a	9.10 a	6.02 b	13.73 a	12.45 c	87.89 c
		C5	15.27 a	8.99 a	6.62 a	13.60 a	14.60 b	83.89 d
		C7	15.30 a	9.00 a	7.01 a	13.60 a	17.22 a	77.81 e
2023	DN428	C0	18.39 a	10.08 a	4.61 d	16.77 a	6.74 e	96.86 a
		C1	18.17 a	9.60 a	4.98 cd	16.65 a	7.78 d	94.33 b
		C3	17.90 a	9.90 a	5.38 c	16.34 a	9.18 c	89.38 c
		C5	17.97 a	9.45 a	6.15 b	16.27 a	10.98 b	84.35 d
		C7	18.06 a	9.34 a	6.89 a	15.81 a	13.15 a	81.66 e
	SJ10	C0	15.75 a	9.52 a	5.84 d	14.26 a	8.62 e	94.73 a
		C1	15.56 a	9.09 a	5.73 d	14.04 a	10.74 d	91.82 b
		C3	15.47 a	9.46 a	6.25 c	13.79 a	12.61 c	87.67 c
		C5	15.26 a	8.91 a	7.45 b	13.72 a	15.07 b	81.95 d
		C7	15.16 a	8.83 a	7.98 a	13.88 a	17.86 a	78.95 e

Table 2. *Cont.*

Year	Variety	Treatment	Panicle Length	Primary Branch Number	Empty Primary Branch Grains	Secondary Branch Number	Empty Secondary Branch Grains	Spikelet Fertility (%)
					F-value			
Year (Y)			0.03	1.44	5.36 *	0.06	0.10	1.93
Variety (V)			480.16 **	26.45 **	23.46 **	339.47 **	16.29 **	4.06 *
CDD (C)			0.20	2.23	11.44 **	0.42	38.26 **	116.83 **
Y×V			0.28	0.64	4.20 *	0.01	0.27	1.86
Y × C			0.20	0.69	0.43	0.18	0.05	3.38
V × C			0.67	0.41	0.85	0.45	4.51 **	2.80
Y × V × C			0.12	0.47	0.30	0.34	0.05	0.06

CDD represents Cold Degree Day (treatment). Lowercase letters (a, b, c, d, e) denote a significance level of $\alpha = 0.05$. Identical letters indicate non-significant differences, whereas different letters indicate significant differences. The numbers and asterisks (*) were obtained through ANOVA analysis, where the numbers represent F-Values and * indicates significance. The symbol * indicates significance at $p < 0.05$. ** indicates significance at $p < 0.01$. NS indicates not significant.

During the grain-filling stage, low-temperature stress had a minor impact on panicle length, primary branch number, and secondary branch number in rice, with no significant differences among treatments. However, with the extension of low-temperature treatment duration, the number of empty grains in primary and secondary branches significantly increased, while spikelet fertility significantly decreased.

3.3.3. Grain Yield and Its Components

The variation trends of yield and component factors obtained by field experiment and pot experiment (Tables 3 and S10) under low-temperature stress at grain-filling stage were consistent and there were significant differences between them only in spikelets per panicle. As shown in Table 2, low-temperature stress during the grain-filling stage had minimal impact on the effective panicle number and spikelets per panicle of DN428 and SJ10, with no significant differences among treatments. However, the differences between varieties were highly significant. Compared to the C0 treatment, low-temperature stress significantly reduced the seed-setting rate, 1000-grain weight, and yield (except for DN428's C1 treatment, where the 1000-grain weight did not differ significantly from the C0 treatment). The reduction intensified as the duration of low-temperature stress increased, reaching its maximum in the C7 treatment. Specifically, for DN428, the decreases in seed-setting rate, 1000-grain weight, and yield were 12.30%, 16.26%, and 32.90%, respectively, in 2022, and 15.13%, 16.36%, and 32.60% in 2023. For SJ10, these decreases were 15.56%, 19.66%, and 36.33% in 2022, and 17.65%, 21.34%, and 38.06% in 2023. In terms of variety, the cold-tolerant variety DN428 exhibits smaller reductions across all indices compared to the cold-sensitive variety SJ10, demonstrating superior cold-tolerance and maintaining greater yield stability under low-temperature stress.

The results indicate (Figure 7) that during the grain-filling stage under low-temperature stress, the rice yield is suppressed. The contribution of yield components for different rice varieties follows the same order of 1000-grain weight > effective panicle number > spikelets per panicle > seed-setting rate, all exhibiting positive direct effects. Among the varieties, DN428 shows a higher yield than SJ10. The contents within the rice grains positively contribute to 1000-grain weight, with starch content having a significantly greater impact than protein content. Low-temperature stress significantly reduced the phloem and vascular bundle areas of the panicle node and leaves (Figure S2), with the largest decreases observed in the C7 treatment (the reductions in phloem and vascular bundle areas for panicle node were 20.11% and 7.65%, respectively, for DN428 and 16.13% and 5.77% for leaves; for SJ10, the reductions were 30.57% and 12.85% for panicle node and

23.35% and 13.78% for leaves). Additionally, the phloem area and vascular bundle area in both panicle stem nodes and leaves positively influence the contribution rate of dry matter translocation. Within the same organ, the impact of the phloem area on the contribution rate of dry matter translocation is greater than that of the vascular bundle area. In terms of these effects, DN428 is less than SJ10.

Table 3. Effect of low-temperature stress on rice yield and yield components during the grain-filling stage.

Year	Variety	Treatment	Effective Panicle Number per Hole	Spikelets per Panicle	Seed-Setting Rate (%)	1000-Grain Weight (g)	Yield (kg/hm^2)
2022	DN428	C0	13.68 a	103.40 a	93.95 a	23.72 a	8241.69 a
		C1	12.77 a	102.92 a	92.71 b	23.63 a	7489.39 b
		C3	12.59 a	101.83 a	88.70 c	22.69 b	6660.33 c
		C5	12.97 a	101.87 a	84.89 d	21.14 c	6274.58 d
		C7	12.62 a	101.97 a	82.40 e	19.87 d	5529.91 e
	SJ10	C0	12.60 a	101.56 a	93.67 a	23.07 a	7033.31 a
		C1	11.96 a	101.05 a	90.16 b	21.87 b	6338.35 b
		C3	11.58 a	100.88	87.61 c	20.79 c	5372.92 c
		C5	11.92 a	100.49 a	82.09 d	19.30 d	5001.43 d
		C7	11.45 a	99.62 a	79.09 e	18.53 e	4477.86 e
2023	DN428	C0	12.99 a	101.58 a	96.20 a	23.40 a	7793.14 a
		C1	12.57 a	101.00 a	93.72 b	23.07 a	7290.37 b
		C3	12.59 a	100.91 a	87.44 c	22.02 b	6417.07 c
		C5	12.77 a	100.75 a	83.90 d	21.14 c	5785.19 d
		C7	12.43 a	100.22 a	81.65 e	19.58 d	5252.36 e
	SJ10	C0	12.83 a	100.68 a	94.61 a	23.15 a	7338.33 a
		C1	12.60 a	100.16 a	90.98 b	21.91 b	6449.29 b
		C3	12.47 a	100.02 a	86.11 c	20.34 c	5520.88 c
		C5	12.70 a	99.97 a	81.46 d	19.23 d	5118.92 d
		C7	12.23 a	99.62 a	77.91 e	18.21 e	4545.47 e
					F-value		
Year (Y)			1.39	12.07 **	0.01	0.41	0.06
Variety (V)			12.69 **	21.26 **	12.39 **	11.33 **	12.02 **
CDD (C)			1.23	2.24	207.95 **	51.30 **	45.75 **
Y × V			9.79 **	2.69	0.02	0.03	0.99
Y × C			0.65	0.39	46.57 **	1.49	0.19
V × C			0.12	0.31	32.11 **	11.88 **	0.55
Y × V × C			0.01	0.22	2.48	0.89	0.14

CDD represents Cold Degree Day (treatment). Lowercase letters (a, b, c, d, e) denote a significance level of $\alpha = 0.05$. Identical letters indicate non-significant differences, whereas different letters indicate significant differences. The numbers and asterisks (*) were obtained through ANOVA analysis, where the numbers represent F-Values and * indicates significance. The symbol ** indicates significance at $p < 0.01$. NS indicates not significant.

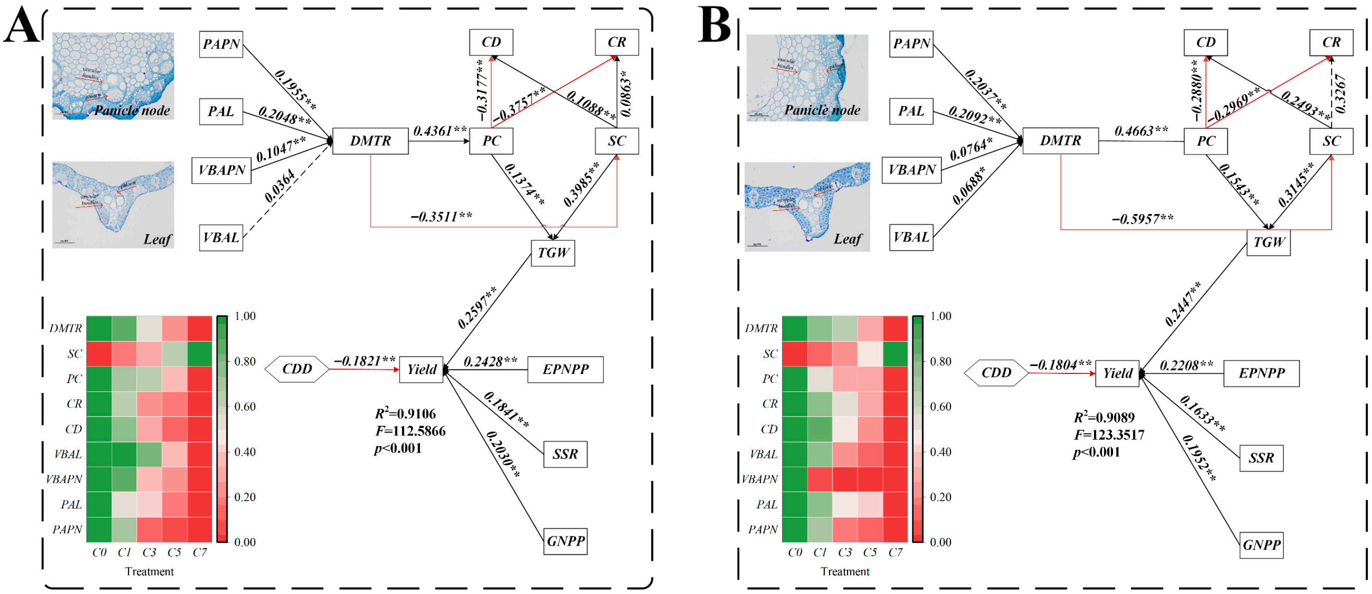

Figure 7. Trends in yield and its components under low-temperature stress during the grain-filling stage and results of ridge regression analysis. (**A**) DN428. (**B**) SJ10. CDD represents Cold Degree Day. PAPN represents the phloem area in the panicle node. PAL represents the phloem area in leaves. VBAPN represents the vascular bundle area in the panicle node. VBAL represents the vascular bundle area in the leaves. DMTR represents the contribution rate of dry matter. PC represents the protein content in grains. SC represents the starch content in grains. CD represents the chalky degree. CR represents the chalky grain rate. TGW represents the thousand-grain weight. EPNPP represents the effective panicle number per plant. SSR represents the seed-setting rate. GNPP represents the grain number per panicle. The values are the standardized coefficients, i.e., path coefficients, calculated through ridge regression analysis. The symbol * indicates significance at $p < 0.05$. ** indicates significance at $p < 0.01$. NS indicates not significant. The black lines represent a positive correlation. The red lines represent a negative correlation. The dashed lines indicate a non-significant correlation.

4. Discussion

4.1. Effects of Low-Temperature Stress on Carbon and Nitrogen Metabolism and Source–Sink Relationships

For efficient resource capture and assimilation exchange, many crops use intricate biological mechanisms to establish a dynamic balance between source organs (like leaves) and sink organs (like seeds) [50,51]. The leaf, as a major source organ, plays a key role in carbon and nitrogen metabolism [52]. Leaves obtain energy through photosynthesis to synthesize carbon dioxide (CO_2) and nitrate ions (NO_3^-) into carbohydrates and amino acids to support crop growth and development [53]. However, abiotic stresses often lead to the disorganization of carbon and nitrogen metabolism, affecting the physiological functions of crops [54–56]. In rice functional leaves under low-temperature stress, this study demonstrated that the activities of nitrogen metabolism-related enzymes, including nitrate reductase (NR), glutamine synthetase (GS), glutamate synthetase (GOGAT), glutamic oxaloacetic transaminase (GOT), and glutamic pyruvic transaminase (GPT), were significantly reduced. These activities intensified as the duration of the low-temperature stress increased, which led to a gradual decrease in the nitrogen accumulation in the leaves. Researchers discovered that nitrogen is one of the primary components of chlorophyll [53] and that nitrogen accumulation in leaves under low-temperature stress is largely determined by the content of photosynthetic pigments like chlorophyll [57]. They also discovered that a reduction in nitrate was faster in dark-colored leaves with sufficient carbohydrates [58]. Reduced nitrogen content in leaves under low-temperature stress will restrict chlorophyll formation and further impede nitrogen uptake, since photosynthesis and chlorophyll concentration in leaves are tightly related [59,60]. In this study, we discovered (Figure 2) that low-temperature stress led to a significant decrease in chlorophyll content in functional leaves and a reduction in the Pn. Additionally, ridge regression analysis confirmed a strong positive relationship between nitrogen accumulation and both chlorophyll content and Pn. The ongoing process of nitrogen assimilation relies on the energy and carbon framework supplied by photosynthesis.

Furthermore, under low-temperature stress, we noted (Figure 3) an upward trend in the activity of enzymes associated with sucrose synthesis, including sucrose synthase (SS) and sucrose phosphate synthase (SPS). Among them, the activities of DN428SS and SPS were higher than those of SJ10, having peaked 5 days earlier than SJ10. With the increase in SS and SPS activities, the trends of sucrose, fructose, and total soluble sugar contents were consistent with the enzyme activities. However, during the same period, the accumulation of sugars in the leaves did not promote the process of nitrogen metabolism. Some studies suggest that carbon and nitrogen assimilate act as signaling molecules involved in the regulation of carbon and nitrogen metabolism [61–63]. Under nitrogen-deficient conditions, carbohydrates accumulate significantly in the leaves in response to low nitrogen stress, and after the nitrogen supply is restored, the carbohydrates flowing into nitrogen metabolism increase [64,65]. Research has confirmed the existence of a feedback regulation mechanism between photosynthetic assimilates and photosynthesis [66–68]. Excessive accumulation of sucrose may inhibit photosynthesis and thus limit nitrogen metabolism through a negative feedback mechanism, which is validated by our results obtained through ridge regression analysis (Figure 2). In addition, it was found that sugar also induced the expression of nitrate reductase genes in Arabidopsis, which we will investigate in more depth in the future.

It is believed that the spike of rice has a certain photosynthetic capacity and its photosynthetic assimilates are mainly applied to its growth [69]. In this study, it was found that under low-temperature stress, the activities of enzymes related to carbon and nitrogen metabolism were significantly reduced in the grains, which led to the limitation of their production capacity and the reduction in carbohydrate content such as starch and nitrogen accumulation. It was found that there were differences in the photosynthetic capacity of grains in different parts of the grain. The grains at the top of the spike (superior grains) were usually exposed to more light and had a relatively strong photosynthetic capacity, so they were able to obtain more nutrients, and their development was better than that of the grains at the bottom (inferior grains) [70]. This study did not determine the effect of low-temperature stress on photosynthesis of the seed grain itself, which may be a direction for further research in the future. The present study was based on the effect of low temperature on the internal carbon and nitrogen metabolism of grains. Under low-temperature stress, the activities of carbon and nitrogen metabolism-related enzymes (AGPase, SSS, SBE, GS, GOGAT, GOT, and GPT) of grains were significantly reduced, which led to the limitation of their own production capacity, and the reduction in starch and other carbohydrates, as well as the accumulation of nitrogen. The difference in production capacity between superior and inferior grains may be related to the unique grain-filling sequence of rice, in which the peaks of carbon and nitrogen metabolism-related enzyme activities of inferior grains were delayed to different degrees compared with those of superior grains, which was ultimately reflected in the delayed response of the peaks of products such as starch and nitrogen accumulation. Different from other carbon and nitrogen metabolism-related enzymes, GBSS and GDH were significantly elevated under low-temperature stress, which still needs further study. In this study, we also found that different parts of the grains were differently sensitive to low-temperature stress, and the inferior grains were more significantly inhibited by the same number of days of low-temperature treatment. For superior grains, short-term low-temperature treatments (1–3 d) reduced the peak value of carbon and nitrogen metabolism-related enzymes, but the arrival time of the peak value was not delayed. In contrast, the peaks of non-structural carbohydrate content and related enzyme activities of inferior grains decreased more significantly under low-temperature stress, and the time to reach the peak was prolonged by 5–10 days (Figure 4). The difference in carbon and nitrogen metabolism response to low temperature was greater in the inferior grains than in the superior grains of the two varieties, and for DN428, the peak of the inferior grains appeared to be shifted back under 5–7 d of low-temperature treatment, whereas it appeared after only 3 d of low-temperature in SJ10.

In addition, low-temperature stress affected the transport of carbon and nitrogen assimilates between source and reservoirs. The researchers found that the phloem, as part of the stream through which photosynthetically assimilated compounds headed by sucrose, amino acids, etc., are transported in both directions from leaves to roots, shoots, and fruits, was impaired under cold conditions. As the spike matures, the grain is transformed into an important reservoir organ, with its own photosynthetic capacity weakened, and the required substances and energy come more from the source organ. The low temperature restricts the transport of streams, leading to a reduction in energy and substances delivered to the grain, reinforcing the existence of superior and inferior grain differences. Due to the differences in the functions of leaves and grains in rice growth, their responses to low temperatures were also very different. The enzyme activities in leaves were increased while those in grains were significantly inhibited by low temperatures, and the nitrogen metabolism-related enzymes in grains were significantly higher than those in leaves, which may be due to the effects of gene expression, and more in-depth research is needed. The fertility period of rice is related to varieties, and rice will move from nutrient growth to reproductive growth under appropriate photoperiod and thermoperiod conditions. Appropriate low-temperature stress is beneficial for rice to pass through vernal flowering; however, low-temperature stress during the grouting and fruiting period significantly reduced the activities of enzymes related to the production of carbon and nitrogen assimilates and delayed the arrival of their peaks, and although the degree of cold stress delayed the time of rice grouting, the low-temperature stress was not significant at the time of the grouting and fruiting period. Under the influence of limited reproductive time, although the low-temperature stress delayed the time of rice filling, at this time, the leaves and stalks went to senescence, and the production and transport capacity was reduced; furthermore, the slow and small amount of material transport could not offset the deficit obtained in the early stage of the grains by the influence of low-temperature stress, so the final yield was lowered.

Based on the aforementioned perspectives, we can infer the reasons for the differences in the trends of sucrose (Suc), fructose (Fru), and total soluble sugar (TSS) content between functional leaves and grains in our experiment, as well as the significant variations in these trends among different varieties. Under low-temperature stress, the content of Suc, Fru, and TSS in superior and inferior grains gradually decreased with the extension of low-temperature treatment duration, which is consistent with the findings of most scholars, indicating that low-temperature stress slows down sugar metabolism and restricts sugar accumulation [71,72]. However, in functional leaves, the trends in the content of Suc, Fru, and TSS showed significant differences among different varieties. For the DN428 variety, low-temperature stress leads to an increase in the content of these sugars, whereas for the SJ10 variety, the content of these sugars only increases during short-term (1–3 days) low-temperature treatment.

We speculate that these differences may be caused by the following two factors:

Synthesis and consumption in source organs: Suc and other substances, as osmotic regulatory substances, increase in content under low-temperature stress, which helps to alleviate the damage to rice cell membranes caused by low-temperature stress [73]. Although low-temperature stress significantly inhibits the net photosynthetic rate (a decrease of 3.19–33.92% 8 days after heading), photosynthesis does not completely stop, and the synthesis of carbohydrates continues. Within a certain period after low-temperature treatment (8–23 days after full-heading for DN428 and 8–28 days after full-heading for SJ10), the activity of key sucrose metabolic enzymes SPS and SS significantly increases (an increase of 5.95–31.29% 8 days after full-heading), and then decreases. Through statistical analysis, we found that the activity of SPS and SS is significantly positively correlated with the content of Suc and other substances, that is, the increase in the activity of SPS and SS helps to form a "buffer mechanism" for sugar synthesis in cells [74]. Meanwhile, according to previous studies, the activity of mitochondrial ATPase and other enzymes related to respiration is severely inhibited under low temperature, leading to a reduction in the consumption of sugars by respiration [75]. This "income-increasing and expenditure-saving" dual regulatory mechanism enables leaves to maintain sugar homeostasis even when photosynthesis is impaired.

The transport of substances between source and sink organs: We found that under low-temperature stress, the vascular bundle area shrinks, which may limit the loading and transport capacity of the phloem, reduce transport efficiency, and thus cause Suc and other substances to linger in source organs such as functional leaves, as well as fail to be transferred to sink organs such as grains in time. This ultimately manifests as an increase in Suc content in functional leaves. It should be particularly noted that the response differences between grains (sink organs) and leaves (source organs) essentially reflect the survival strategy of plants to prioritize maintaining the function of source organs under stress. This tissue-specific regulatory mechanism may be an important adaptive strategy for rice to cope with short-term low-temperature stress. We are currently conducting proteomic and metabolomic analyses to further elucidate this regulatory network. Compared with SJ10, DN428 is less affected by low temperature in terms of TSS accumulation, which reflects its stronger cold-tolerance. In the future, we plan to conduct multi-omics research to further explore the reasons for the increase in TSS content in the functional leaves of DN428.

4.2. Effects of Carbon and Nitrogen Metabolism Disorders on Yield

The effects of low-temperature stress on carbon and nitrogen metabolism in rice are multilayered and multifaceted, and will eventually be reflected in yield. Low-temperature stress adversely affected rice productivity, and this study found that the proportion of dry matter distribution to the grain as well as the theoretical weight of superior and inferior grains, maximum grouting rate, and average grouting rate under low-temperature stress decreased significantly with the increase in low-temperature treatment time, thus affecting the formation of the fruiting rate and 1000-grain weight, and the yield was significantly reduced under the influence of simultaneous reduction in the rate of fruiting and 1000-grain weight (except for the treatment of DN428 C1 treatment). The yield was significantly reduced under the influence of simultaneous reduction in fruiting rate and thousand-grain weight (except for DN428 C1 treatment) and the reduction in fruiting rate, thousand-grain weight, and yield of DN428 was smaller than that of SJ10, which was less affected, while the number of grains per spike was not significantly affected by the low temperature.

The effects of low-temperature stress on rice yield during the grain-filling stage were mainly twofold: first, photosynthesis was blocked, and the reduction in photosynthetic products led to the reduction in synthesis of starch precursors [76]; meanwhile, nitrogen, which is an important element for photosynthesis and starch synthesis, was further inhibited by the reduction in its accumulation in functional leaves [77], and the activity of key enzymes of starch synthesis was also inhibited, leading to the inhibition of starch synthesis and the reduction in starch production. Key enzyme activities were also inhibited, resulting in reduced starch synthesis, and since starch is the most abundant substance in rice grains, impaired starch biosynthesis will, to some extent, lead to a reduction in rice thousand kernel weight [78], so effective carbohydrate metabolism and starch synthesis are necessary for rice grain development. Secondly, anthers require a specific temperature range during development, and low temperature affects the morphology of rice anther cell organization, resulting in poor anther development, reduced anther dehiscence coefficient, lower pollination rate, etc. [79,80], which ultimately lead to an increase in vacuolated grains and a decrease in the rate of fruiting. Previous studies have found that experiencing low-temperature and cold damage in rice during the filling and fruiting stage can lead to a decrease in the maximum filling rate and delay the entire filling process, which in turn causes a decrease in the fruiting rate and thousand-grain weight, resulting in a reduction in rice yield [81–83], and the present study, through ridge regression analyses, found that there were differences between DN428 and SJ10 in the yield components that had the most significant direct effect on yield, which were the effective number of spikes per plant and the thousand-grain weight, respectively. It was previously suggested that rice spikes also have photosynthetic capacity, which is also essential for yield formation, and their photosynthetic products are preferentially used to satisfy the growth and development of their tissues [84,85], i.e., the higher the effective number of spikes in a single plant, the more fully the light energy is utilized, and the higher the potential yield capacity, which is one of the reasons why the yield of DN428 is higher than that of SJ10. It has been found that low-temperature stress delays the period of rice maturity, and we similarly observed a significant delay in maturity postponement under prolonged low-temperature treatments (5 to 7 d) during harvest; however, the prolongation of days of plumping did not result in an increase in yield. The fertility period of rice varies from variety to variety, and under suitable light and temperature conditions, rice shifts from nutritive to reproductive growth. Moderate low-temperature stress helped rice to survive vernalization, but low-temperature stress during the grouting and fruiting stage significantly reduced the activities of enzymes related to carbon and nitrogen assimilates and delayed their peak times. Although low-temperature stress prolonged the filling time of rice during the limited reproductive period, the leaves and stalks tended to be senescent at this time, and their production and transport capacities were significantly reduced. The slow and small amount of material transport could not make up for the deficit caused by low-temperature stress in the pre-grain stage, which ultimately led to lower grain yield.

5. Conclusions

Compared to normal temperature conditions, prolonged low-temperature stress during the grain-filling stage significantly inhibits the activity of nitrate reductase (NR), glutamine synthetase (GS), glutamate synthase (GOGAT), glutamate dehydrogenase (GDH), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), adenosine diphosphate glucose pyrophosphorylase (AGPase), granule-bound starch synthase (GBSS), soluble starch synthase (SSS), and starch branching enzyme (SBE) in rice, consequently impeding photosynthetic efficiency. Furthermore, low temperatures lead to a reduction in the area of vascular bundles and phloem, limiting the synthesis and translocation of carbon and nitrogen metabolites. These changes not only delay the peak timing of various physiological indicators but also result in insufficient grain-filling, ultimately constraining yield improvement. The decline in various indicators becomes more pronounced with extended exposure to low temperatures. Within the same variety, superior grains demonstrate greater stress resistance compared to inferior grains, exhibiting smaller decreases across various indicators. Notably, leaves and grains display differential response mechanisms to low-temperature stress. In comparison to SJ10, the DN428 variety exhibits superior performance across various indicators with smaller decreases, reflecting enhanced cold-tolerance and a greater capacity to maintain stable yields.

Future research should integrate multi-omics technologies to systematically decipher the molecular regulatory networks underlying the inhibition of key enzyme activities under low-temperature stress, encompassing promoter methylation regulation, transcription factor interaction networks, and post-translational modification mechanisms. Through comparative genomics and epigenomics analyses, particular emphasis should be placed on identifying core regulatory elements responsible for cold-tolerance divergence between DN428 and SJ10.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy15020417/s1: Figure S1: Photos from trials; Figure S2: The area of vascular bundles and phloems in the panicle nodes and functional leaves; Table S1: Effects of low-temperature stress during the grain-filling stage on the field experiment and pot experiment of photosynthesis-related indices in functional leaves of rice; Table S2: Effects of low-temperature stress during the grain-filling stage on the variance values of photosynthesis-related indices in functional leaves of rice; Table S3: Effects of low-temperature stress during the grain-filling stage on the variance values of total soluble sugar content and sucrose metabolism-related enzyme activities in functional leaves of rice; Table S4: Effects of low-temperature stress during the grain-filling stage on the variance values of total soluble sugar content in superior and inferior grains of rice; Table S5: Effect of low-temperature stress during the grain-filling stage on the variance values of amylum content and amylum metabolism-related enzyme activities in superior grains of rice; Table S6: Effect of low-temperature stress during the grain-filling stage on the variance values of amylum content and amylum metabolism-related enzyme activities in inferior grains of rice; Table S7: Effects of low-temperature stress during the grain-filling stage on the variance values of nitrogen accumulation and related enzyme activities in functional leaves of rice; Table S8: The effect of low-temperature stress during the grain-filling stage on the variance values of nitrogen accumulation and related enzyme activities in superior grains of rice; Table S9: The effect of low-temperature stress during the grain-filling stage on the variance values of nitrogen accumulation and related enzyme activities in inferior grains of rice; Table S10: Effect of low-temperature stress on rice yield and yield components during the grain-filling stage pot experiment.

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