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Effects of Elevated CO₂ Concentration and Nitrogen Application Levels on the Accumulation and Translocation of Non-Structural Carbohydrates in Japonica Rice

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Received: 5 May 2020; Accepted: 1 July 2020; Published: 3 July 2020

Abstract: Non-structural carbohydrates (NSC) play an important role in yield formation. In this paper, the relationships of NSC accumulation and translocation with yield formation were investigated under elevated CO₂ concentrations ([CO₂]) and nitrogen (N) application rates. A japonica rice (*Oryza sativa* L.) cultivar, “Nanjing 9108,” was grown at three [CO₂]-Ambient (T0), ambient + 160 μmol·mol⁻¹ (T1), and ambient + 200 μmol·mol⁻¹ (T2)—in open-top chambers (OTC), with three levels of N fertilizer application rates—10 gN·m⁻² (N1), 20 gN·m⁻² (N2), and 30 gN·m⁻² (N3)—Which were set in OTCs using pot experiments. The results showed that the concentration of NSC (C_{NSC}) and the total mass of NSC stored in stems (TM_{NSC}) under T1 and T2 were higher than those in the T0 treatment, and the C_{NSC} and TM_{NSC} of N3 were lower than those of N1 and N2 at the heading stage. The C_{NSC} and the TM_{NSC} were significantly positively correlated with the stem biomass during the growth period and were notably negatively correlated with the N content in leaves (N_{leaf}) at the heading and filling stages. The seed setting rate was significantly positively related to the apparent transferred mass of NSC from stems to grains (ATM_{NSC}) at the filling stage, while the relationship between yield and the ATM_{NSC} was not statistically significant. Although there was no difference in the apparent contribution of transferred NSC to grain yield (AC_{NSC}) between treatments, NSC stored in stems further accumulated obviously during the late filling stage, implying that the grain yield of “Nanjing 9108” was predominantly sink-limited. We concluded that elevated [CO₂] improved the concentration of NSC at the rice heading stage. The interaction between elevated [CO₂] and N fertilizer rates significantly influenced the concentration of NSC at the filling stage. Rice stems NSC reaccumulated at the late grain filling stage, which implies a restriction on NSC transference to grain.

Keywords: rice; elevated CO₂ concentration; nitrogen application levels; stems; non-structural carbohydrates; accumulation; translocation

1. Introduction

With the rapid development of the world’s industry and economy, the CO₂ concentration ([CO₂]) has increased from 278 μmol·mol⁻¹ before the industrial revolution to 414 μmol·mol⁻¹ [1] and is

predicted to reach 538–670 $\mu\text{mol}\cdot\text{mol}^{-1}$ in 2100 under the greenhouse gas emission stabilization scenario [2]. As the raw material for plant photosynthesis, changes in $[\text{CO}_2]$ will inevitably affect the growth and development of plants and will further influence the accumulation and distribution of their assimilation [3–5]. Synthetic N fertilizers are widely used in agricultural ecosystems. Worldwide, the N use efficiency of cereal production is approximately 33% [6]. By optimizing N fertilizer management, reducing the current N application rates by 30–60% would still maintain crop yields and N balance in rotations, which could contribute to restoring, enhancing, and protecting degraded environments [7].

Rice, the world's most important cereal crop that serves as a food resource for more than half the world's population, accounts for 35–75% of the calories consumed by more than 3 billion Asians [8,9]. China is the world's largest producer and consumer of rice. It is expected that global crop demand will increase by 100–110% from 2005 to 2050 to meet the growing population [10]. However, in the past ten years, the annual growth in rice yield has fallen below 1% and is virtually nil in Asia [11]. Moreover, abiotic stresses, such as drought, flooding, and abnormal temperatures also challenge current and future global rice production [8,12–14]. The compensation of CO_2 fertilization represents an attractive path to increase crop yield, narrow existing yield gaps, and, hence, will contribute to sustainable food production in the coming decades [15]. Non-structural carbohydrates (NSC) are the main products of photosynthesis and the substrates for respiration, and are essential for plant growth and energy metabolism [16]. NSC stored in stems before heading can be translocated into the panicle to supply grain filling [17,18], which contributes 1–30% to yield [19,20]. The use and supply of NSC are considered adjustments between sources and sinks, and studies have provided evidence on whether the grain yield in cereals is source- or sink-limited [21,22]. Moreover, pre-anthesis NSCs reserved in stems are closely related to the sink strength and seed setting rate of inferior spikelets [23] and play an important role in improving the lodging resistance of rice [24,25]. Studies have shown that elevated $[\text{CO}_2]$ significantly increased the NSC content in rice stems [26–28] and leaves [29]. Low N application increased stem NSC accumulation before the heading stage and NSC translocation during the grain filling stage, and also increased the seed setting rate of superior spikelets [18]. The relative contribution of pre-anthesis NSC to total NSC in grains was higher at low N than at high N [30]. Excessive N application delayed plant senescence, further reducing NSC translocation from stems to grains [31].

More is known about the effects of a single factor, such as elevated $[\text{CO}_2]$ [26,28], N fertilizer rates [20,32], and water conditions [33,34], or the interaction between N fertilizer rates and water conditions [31,35] on NSC accumulation and translocation in grain development, leaving the effects of elevated $[\text{CO}_2]$ combined with N rates unclear. In light of previous studies [18,20,26–28], we hypothesize that elevated $[\text{CO}_2]$, N fertilizer rates, and their interactions all affect the accumulation and translocation of rice stem NSC and, hence, yield formation. To test this hypothesis, we measured the rice's aboveground biomass, as well as its components (stem, leaf, spike), the NSC content in stems, and the N content in leaves during the main growth period and also quantified the contribution of NSC stored in stems before heading to grains under two elevated $[\text{CO}_2]$ levels and three N fertilizer rates. In addition, transplanting dates are important cultivation factors for rice production. Different dates of transplanting with respect to photoperiodic and thermal regimes [36,37], as well as the length of the growth duration of rice [38], which consequently have great influence on the phasic development and partitioning of dry matter. In this study, transplanting dates treatment was employed to further understand the transfer of NSC from stem to grain. Our study will help to illuminate the relationships of NSC accumulation and translocation with yield formation in rice under different $[\text{CO}_2]$ and N fertilizer levels, and will provide a scientific basis for optimizing N management and maintaining stable rice yields under the background of global climate change.

2. Materials and Methods

2.1. Experimental Design

2.1.1. ([CO₂]) Enrichment and N Fertilizer Treatments

The experiment was conducted at the increasing [CO₂] experimental platform at the Nanjing University of Information Science & Technology (32.21° N, 118.72° E), Jiangsu Province, China, during the rice growing season from May to October, 2019. The region belongs to the humid subtropical climate zone, with a mean annual temperature of 15.6 °C and mean annual precipitation of about 1100 mm. The platform consisted of 12 open-top chambers (OTCs) and a [CO₂] control system. The OTCs were regular octagonal prism-shaped, 3 m high, and had a bottom area of approximately 12 m². CO₂ sensors (GMM222, Vaisala Inc., Helsinki, Finland) were suspended in the middle of each chamber, 1.5 m above the ground, to detect the [CO₂] in real time, and circular PVC tubes covered with small holes on the surface, fixed 1.0 m above the ground, acted as gas supply systems around each chamber. We used an automatic [CO₂] control system to maintain the target concentrations of treatments based on the monitoring of GMM222 CO₂ sensors during the rice growing season. The control system was computer programmed, operated for 24 h per day, and adjusted the supply CO₂ in the treatments every 10 s. The [CO₂], air temperature, relative humidity, soil temperature, and humidity inside and outside the OTCs were monitored in real time and viewed online.

In this experiment, [CO₂] was set at three levels: ambient (T0), ambient + 160 µmol·mol⁻¹ (T1), and ambient + 200 µmol·mol⁻¹ (T2). Four OTCs as replicates per treatment were laid out. In total, there were 12 pots for each N fertilizer level per OTC. Pots treated with the same N level were put into plastic crates to prevent nitrogen movement among treatments. The CO₂ supply for T1 and T2 was conducted from 21 June to 9 November, 2019. The average [CO₂] of the T0, T1, and T2 treatments was 435 ± 18, 569 ± 23, and 615 ± 27 µmol·mol⁻¹, respectively. The N application treatment was performed using pots with an 18 cm upper diameter, 14.5 cm lower diameter, and 18 cm height. The N fertilizer levels were 10 g N·m⁻² (N1), 20 g N·m⁻² (N2), and 30 g N·m⁻² (N3). The soil in pots was consistent with that in the field outside the OTCs, and the soil type was waterloggogenic paddy soil with the following properties at 0–20 cm depth: pH of 6.1, soil organic carbon of 11.66 g·kg⁻¹, total N of 1.24 g·kg⁻¹, total P of 0.90 g·kg⁻¹, and total K of 19.50 g·kg⁻¹. The N application for each treatment was split into three different doses. The N1 treatment received 60% as basal fertilizer, 20% as tillering fertilizer, and 20% as panicle fertilizer. The N2 and N3 treatments received 40% as basal fertilizer, 40% as tillering fertilizer, and 20% as panicle fertilizer (in 2018, it was suspected that high basal fertilizer application in the N3 treatment caused seedlings to wilt or even die. Therefore, the proportion of basal fertilizer in the N2 and N3 treatments in 2019 was adjusted to 40%).

2.1.2. Pot and Field Experiments

Conventional japonica rice (Nanjing 9108), which is widely cultivated in Jiangsu Province, China, with a full growth period of 149–153 days, was employed in this study. Rice that grew in the OTCs was sown on 21 May and transplanted on 20 June 2019, with three seedlings per pot. The main growth stages and fertilizer management practices are shown in Table 1. Weeds, insects, and diseases were controlled by using standard herbicides and pesticides to prevent yield loss.

To investigate the response of NSC accumulation and translocation to different dates of transplanting, three transplanting date treatments were set for field rice—June 20 (SD1), June 30 (SD2), and July 10 (SD3)—Using 30-day-old seedlings. There were also three seedlings per hill, and the planting density was 20 hills·m⁻², with 30.0 cm row spacing and 16.7 cm intra-row spacing. The harvest dates of SD1, SD2, and SD3 were 25 October, 26 October, and 28 October, respectively. Field management practices, such as water supply and fertilizer applications, were consistent with local practices.

Table 1. Main growth stages and management.

Date	Growth Stage	Management
19 June 2019	Tillering	Compound fertilizer, N: P: K: 15%:15%:15%, accounting for 60% (N1), 40% (N2, N3) of total N application
20 June 2019		Transplanting
5 July 2019		Urea, accounting for 20% (N1), 40% (N2, N3) of total N application
21 July 2019		Urea, accounting for 20% of total N application
26 August 2019		
21 October 2019	Maturity	

2.2. Biomass Sampling and Measurements

At the heading stage (defined as the date when approximately 50% of panicles in a canopy had emerged), rice plants with the same growth status (plant height and ear growth status, etc.) were marked. Five marked rice plants in the N fertilizer treatments (pot) were selected from each OTC and were cut off at the soil surface at the heading, filling (20 days after heading), and maturity stages. Nine marked rice plants (three replicates) per transplanting date treatment in the field were collected every 7 days from the heading stage.

The aboveground biomass was separated into six components: the top three leaves, remaining leaves, stems (including culms and sheaths), cobs, empty grains, and full grains. The remaining plants in each sampling pot were harvested and divided into leaf blades, stems, and ears. All samples were placed in an oven for 30 min at 105 °C, dried at 80 °C to constant weight, and weighed to obtain the biomass of each organ.

2.3. Extraction and Determination of Nonstructural Carbohydrates (NSC)

The soluble sugar and starch contents in rice stems were determined by the anthrone–H₂SO₄ method [39]. The dried rice stem samples were ground with a pulverizer, sieved through a 60-mesh sieve, and weighed to 100 mg per sample into 15 mL centrifuge tubes. Ten milliliters of 80% ethanol were added to the centrifuge tube and extracted at 80 °C in a water bath for 30 min. After the removal and cooling to room temperature, the tube was centrifuged at 3500× g for 10 min, and the supernatant was collected. The extraction was repeated twice as described above; the supernatant was combined three times and brought to 25 mL for the determination of the soluble sugar content. Then, the residue was dried, 2 mL distilled water was added, followed by gelatinization in a boiling water bath for 15 min. Then, 2 mL 9.2 mol·L^{−1} HClO₄ was added and stirred after cooling. After 15 min of extraction, 6 mL distilled water was added and centrifuged at 4000× g for 10 min, and then the supernatant was collected. HClO₄ (2 mL, 4.6 mol·L^{−1}) was further added to the centrifuge tube, stirred and extracted for 15 min. Eight milliliters of distilled water was added, mixed, and centrifuged for 10 min. The two supernatants were combined and made up to 50 mL with distilled water for the determination of the starch content. Three replicates were measured for each N level in the same OTC. The NSC of field rice was measured twice for each replicate.

2.4. Measurement of the N Content in Leaves (N_{leaf})

The top three leaves were pulverized with a grinder and sieved through a 100-mesh sieve. The N content was determined by an elemental analyzer (CHNOS Elemental Analyzer vario El III, Germany, SEAL Analytical GmbH). Three replicates were measured for each N level in the same OTC. The N content of field rice was measured twice for each replicate.

2.5. Definition of Abbreviations

In this study, the NSC refers to starch and soluble sugars in stems. Therefore, the C_{NSC} (mg·g^{−1}) was estimated by summing the concentrations of sugars and starch. Referring to Li et al. [18],

Pan et al. [20], and Wu et al. [40], the total mass of NSC stored in stems (TM_{NSC} , g) was calculated as stem biomass multiplied by the corresponding C_{NSC} . The apparent transferred mass of NSC from stems to grain (ATM_{NSC} , g) was calculated as the TM_{NSC} at heading minus at maturity (Equation (1)). The apparent contribution of transferred NSC to grain yield (AC_{NSC} , %) was defined as the ratio of ATM_{NSC} to the corresponding grain yield (Equation (2)).

$$ATM_{NSC} = \text{total mass of NSC in stem at heading} - \text{total mass of NSC in stem at maturity} \quad (1)$$

$$AC_{NSC} = ATM_{NSC} / \text{grain yield} \times 100 \quad (2)$$

The seed setting rate (%) was defined as the number of filled grains divided by the number of total grains per panicle. Details of abbreviations are listed in Table 2.

Table 2. List of abbreviations, description and units.

Abbreviation	Description and Unit
N_{leaf}	Nitrogen (N) content in leaves, $\text{mg} \cdot \text{g}^{-1}$
NSC	Non-structural carbohydrates
C_{NSC}	The concentration of NSC, $\text{mg} \cdot \text{g}^{-1}$
TM_{NSC}	The total mass of NSC stored in stems, g
ATM_{NSC}	The apparent transferred mass of NSC from stems to grains, g
AC_{NSC}	The apparent contribution of transferred NSC to grain yield, %

2.6. Statistical Analysis

All the statistical analyses were performed using the SPSS 17.0 statistical software package (SPSS, Chicago, IL, USA). A two-way ANOVA was used to test the effects of elevated CO_2 , N fertilizer levels, and the interaction between $[\text{CO}_2]$ and N. The one-way ANOVA was applied to test the difference of a testing item between treatments. The means were compared using the least significant difference (LSD). Linear regression was used to model the relationship between two items.

3. Results

3.1. Effects of Elevated $[\text{CO}_2]$ and N Fertilizer Levels on N_{leaf}

Elevated $[\text{CO}_2]$ significantly affected N_{leaf} at the heading and filling stages (Figure 1a,b). The N_{leaf} treated with elevated $[\text{CO}_2]$ was lower than that of T0. At the heading stage, the N_{leaf} of T1N2 (indicating that the $[\text{CO}_2]$ is T1, the N fertilizer level is N2, and the same below) and T2N2 was significantly lower than that of T0N2 by 10.8% and 13.9% ($p < 0.01$), respectively. At the filling stage, T1N1 and T2N1 were lower than T0N1 by 12.1% and 19.1% ($p < 0.05$), respectively, T2N2 was lower than T0N2 by 13.5% ($p < 0.01$), and T1N3 was lower than T0N3 by 18.2% ($p < 0.01$). Elevated $[\text{CO}_2]$ had no significant effect on N_{leaf} at the mature stage (Figure 1c), while only T2N3 significantly increased by 15.9% compared with T1N3 ($p < 0.05$).

N fertilizer levels had a significant impact on N_{leaf} in all three growth stages (Figure 1). At the heading stage, N_{leaf} increased with increasing N fertilizer level, but there was no distinct difference between N2 and N3 under the T0 treatment or between N1 and N2 levels under the T1 and T2 treatments (Figure 1a). At the filling stage, N_{leaf} was significantly different among the three N fertilizer levels under the T0 and T2 treatments, shown as $N3 > N2 > N1$, but there was no distinct difference between T0N2 and T0N3. Under the T1 treatment, the N_{leaf} of N2 was significantly higher than that of N1 by 14.5% ($p < 0.05$). At the mature stage, the N_{leaf} of N3 was significantly higher than that of N1 and N2 (Figure 1c). The interaction between elevated $[\text{CO}_2]$ and N fertilizer levels was not striking in all three growth stages (Figure 1).

Figure 1. Effects of elevated $[\text{CO}_2]$ and N fertilizer levels on N_{leaf} at the heading (a), filling (b), and mature (c) stages. Three different levels of $[\text{CO}_2]$ and N fertilizer application were considered. For $[\text{CO}_2]$: T0 (ambient), T1 (ambient + $160 \mu\text{mol}\cdot\text{mol}^{-1}$), and T2 (ambient + $200 \mu\text{mol}\cdot\text{mol}^{-1}$). For N fertilizer: N1 ($10 \text{ g N}\cdot\text{m}^{-2}$), N2 ($20 \text{ g N}\cdot\text{m}^{-2}$), and N3 ($30 \text{ g N}\cdot\text{m}^{-2}$). The error bar represents the SE ($n = 12$). Same uppercase letters indicate no significant effects ($p > 0.05$) of $[\text{CO}_2]$ on N_{leaf} at the same N fertilizer level, while same lowercase letters indicate no significant effects ($p > 0.05$) of N fertilizer levels on N_{leaf} at the same $[\text{CO}_2]$.

3.2. Effects of Elevated $[\text{CO}_2]$ and N Fertilizer Levels on the Accumulation and Translocation of NSC

Elevated $[\text{CO}_2]$ and N fertilizer levels had significant effects on C_{NSC} at the heading stage (Figure 2a). The C_{NSC} treated with elevated $[\text{CO}_2]$ was significantly higher than that of T0 at the same N fertilizer level ($p < 0.05$). The C_{NSC} of T1 treatment increased by 15.7–26.8%, and increased by 11.5–31.4% of the T2 treatment ($p < 0.05$) compared with the T0 treatment. The C_{NSC} of N3 was lower than that of N1 and N2 at the same $[\text{CO}_2]$. There was no significant difference among the different N levels under the T0 treatment, but N1 and N2 were significantly higher than N3 under the T1 and T2 treatments ($p < 0.05$). The interaction between elevated $[\text{CO}_2]$ and the N fertilizer level was not striking.

At the filling stage, the C_{NSC} of the T2 treatment was significantly higher than that of T0 and T1 at N1 ($p < 0.05$), the C_{NSC} treated with elevated $[\text{CO}_2]$ was significantly higher than that of T0 at N2 ($p < 0.01$), and the C_{NSC} of T1 treatment was significantly higher than that of T0 and T2 at N3 ($p < 0.01$). Under the T0 and T2 treatments, the C_{NSC} of N3 was significantly lower than that of N1 and

N2 ($p < 0.01$). Under the T1 treatment, the C_{NSC} of N1 was significantly lower than that of N2 and N3 ($p < 0.05$). The interaction between elevated $[CO_2]$ and N fertilizer level was significant ($p = 0.000$).

Figure 2. Effects of elevated $[CO_2]$ and N fertilizer levels on C_{NSC} at the heading (a), filling (b), and mature (c) stages. The error bar represents the SE (n = 12). Same uppercase letters indicate no significant effects ($p > 0.05$) of $[CO_2]$ on C_{NSC} at the same N fertilizer level, while same lowercase letters indicate no significant effects ($p > 0.05$) of N fertilizer levels on C_{NSC} at the same $[CO_2]$. Symbols are the same as in Figure 1.

At the mature stage, only the C_{NSC} of the T1N3 treatment was significantly lower than that of T1N1 and T0N3 ($p < 0.05$), and the C_{NSC} of T0N2 treatment was significantly lower than that of T0N3 ($p < 0.05$) (Figure 2c).

The effect of elevated $[CO_2]$ on TM_{NSC} did not reach a significant level overall (Table 3). At the heading stage, the TM_{NSC} under elevated $[CO_2]$ was higher than that of T0 at the same N fertilizer level. Only the TM_{NSC} of the T2N2 treatment was significantly higher than T0N2 by 70.6% ($p < 0.01$). Under the same $[CO_2]$, the TM_{NSC} of N3 was lower than that of N1 and N2 at the heading stage, and the TM_{NSC} of T2N2 was significantly higher than T2N3 by 59.1% ($p < 0.05$). The TM_{NSC} of T2N3 was significantly lower than that of T2N1 and T2N2 ($p < 0.05$) at the filling stage. There was no significant difference among treatments at the mature stage. The interaction between elevated $[CO_2]$ and N fertilizer level was not significant in all three growth stages (Table 3).

Table 3. Effects of elevated [CO₂] and N fertilizer levels on TM_{NSC} (g·5 plants^{−1}), ATM_{NSC} (g·5 plants^{−1}), and AC_{NSC} (%).

CO ₂	N	TM _{NSC}			ATM _{NSC}		AC _{NSC}	
		Heading	Filling	Maturity	Heading to Filling ³	Heading to Maturity ⁴	Heading to Filling	Heading to Maturity
T0	N1	2.7 ± 0.4 ¹ Aa ²	1.5 ± 0.2 Aa	2.9 ± 0.2 Aa	1.2 ± 0.5 Aa	−0.2 ± 0.5 Aa	10.8 ± 4.3 Aa	−1.5 ± 3.5 Aa
	N2	2.5 ± 0.6 Ba	1.6 ± 0.2 Aa	2.5 ± 0.2 Aa	0.9 ± 0.6 Aa	0.0 ± 0.4 Ba	9.0 ± 6.0 Aa	−0.4 ± 2.4 Ba
	N3	2.4 ± 0.2 Aa	1.0 ± 0.3 Aa	2.7 ± 0.2 Aa	1.4 ± 0.3 Aa	−0.2 ± 0.1 Aa	11.2 ± 2.9 Aa	−1.4 ± 0.3 Aa
T1	N1	3.8 ± 0.6 Aa	1.2 ± 0.4 Aa	2.7 ± 0.5 Aa	2.6 ± 0.9 Aa	1.0 ± 0.7 Aa	22.9 ± 8.4 Aa	7.9 ± 4.8 Aa
	N2	3.6 ± 0.3 ABa	2.7 ± 0.7 Aa	2.6 ± 0.1 Aa	0.9 ± 1.0 Aa	1.0 ± 0.2 Aa	9.2 ± 8.0 Aa	6.0 ± 0.9 Aa
	N3	3.0 ± 0.1 Aa	1.90.5 Aa	2.6 ± 0.4 Aa	1.1 ± 0.5 Aa	0.3 ± 0.3 Aa	8.6 ± 4.0 Aa	2.6 ± 2.1 Aa
T2	N1	3.9 ± 0.6 Aab	2.2 ± 0.3 Aa	3.0 ± 0.4 Aa	1.7 ± 0.4 Aa	0.9 ± 0.6 Aab	13.2 ± 3.1 Aa	5.9 ± 3.8 Aab
	N2	4.2 ± 0.2 Aa	2.5 ± 0.2 Aa	2.7 ± 0.2 Aa	1.8 ± 0.1 Aa	1.5 ± 0.1 Aa	15.1 ± 0.9 Aa	9.9 ± 0.8 Aa
	N3	2.7 ± 0.3 Ab	0.9 ± 0.4 Ab	2.9 ± 0.2 Aa	1.8 ± 0.6 Aa	−0.2 ± 0.2 Ab	17.1 ± 5.8 Aa	−1.1 ± 1.0 Ab
Different source and p value	CO ₂	0.007	0.150	0.693	0.503	0.012	0.544	0.008
	N	0.046	0.012	0.667	0.451	0.039	0.565	0.057
	CO ₂ × N	0.483	0.100	0.997	0.498	0.427	0.452	0.334

¹ Values are the means ± SE (n = 12). ² Same uppercase letters in the same column indicate no significant effects ($p < 0.05$) of [CO₂] at the same N fertilizer level, while same lowercase letters in the same column indicate no significant effects ($p < 0.05$) of N fertilizer levels at the same [CO₂]. ³ Values are the difference between the heading stage and filling stage. ⁴ Values are the difference between the heading stage and maturity.

The TM_{NSC} at the filling stage was extremely significantly lower than that at the heading stage ($p < 0.01$) but rebounded at the mature stage (Table 3). The ATM_{NSC} and AC_{NSC} from heading to maturity were significantly lower than those from heading to filling ($p < 0.01$), which may have been due to the stem NSC accumulating again at the mature stage (Table 3). The effects of elevated [CO₂] and N fertilizer levels on ATM_{NSC} and AC_{NSC} were not significant. At the same N fertilizer level, both ATM_{NSC} and AC_{NSC} from heading to maturity under elevated [CO₂] were higher than those of T0. The ATM_{NSC} and AC_{NSC} of T1N2 and T2N2 were significantly higher than those of T0N2 ($p < 0.05$), and that of T2N2 was significantly higher than of T2N3 ($p < 0.01$).

3.3. Effects of Transplanting Dates on the Accumulation of NSC

The dynamics of NSC and its components after heading in the transplanting date treatments were consistent (Figure 3). Overall, the soluble sugar content, starch content, C_{NSC}, and TM_{NSC} all decreased initially and then increased. Except for the starch content, C_{NSC}, and TM_{NSC} of SD2, the other components all rebounded to the heading stage level 56 days after heading (DAH) (Figure 3). The NSC components of SD1 and SD2 were reduced to the minimum 21 DAH and remained stable 21–28 DAH; those of SD3 were minimal 28 DAH (Figure 3). Subsequently, the C_{NSC} and TM_{NSC} increased obviously for the three transplanting dates (Figure 3c,d). This was mainly due to the sharp increase in the soluble sugar content at this time (Figure 3a), while the starch content gradually increased 42 DAH (Figure 3b). This phenomenon is consistent with the data reported in Table 3.

3.4. Relationships among Stem Biomass, N_{leaf}, and C_{NSC}

The regression analysis indicated that the C_{NSC} in potted rice stems during the growing season was positively correlated with stem biomass (Figure 4). According to the slope of the linear equation, C_{NSC} increased by approximately 13.5 mg·g^{−1}, 30.2 mg·g^{−1}, and 10.6 mg·g^{−1} for each 1 g increase in stem biomass at the heading, filling, and mature stages, respectively (Figure 4).

Figure 3. Effect of transplanting date on the soluble sugar content (a), starch content (b), C_{NSC} (c) and TM_{NSC} (d) in rice. The SD1, SD2, and SD3 refer to rice transplanting date on 20 June, 30 June, and 10 July, respectively. The error bar represents the SE ($n = 6$). Same lowercase letters indicate no significant effects ($p > 0.05$) of transplanting dates on the NSC in rice. DAH is days after heading.

Figure 4. Relationships between the C_{NSC} and stem biomass of potted rice.

Further analysis showed that the C_{NSC} and TM_{NSC} of potted rice decreased with the increase in N_{leaf} at the heading and filling stages (Figure 5), but the relationship was not significant at the mature stage (data not shown). For every $10 \text{ mg}\cdot\text{g}^{-1}$ increase in N_{leaf} , TM_{NSC} decreased by 2.3 g and 1.7 g at the heading and filling stages ($p < 0.01$), respectively (Figure 5b), indicating that higher N_{leaf} was not conducive to the accumulation of stem NSC.

Figure 5. Relationship between the C_{NSC} (a), the TM_{NSC} (b), and the N_{leaf} of potted rice.

3.5. Relationship between ATM_{NSC} and Yield Components

The seed setting rate, grain weight, and ATM_{NSC} of potted rice at the filling stage were analyzed. The results showed that there was a noteworthy positive linear correlation between the seed setting rate and ATM_{NSC} . The larger the ATM_{NSC} , the more stem NSC transferred to grains and the higher the seed setting rate (Figure 6a). The correlation between ATM_{NSC} and grain weight was not significant (Figure 6b).

Figure 6. Relationship between seed setting rate (a), grain weight (b), and the ATM_{NSC} of potted rice.

Further regression analysis of the seed setting rate, grain weight, and ATM_{NSC} of field rice from 0–21 DAH showed that the larger the ATM_{NSC} , the higher the seed setting rate and grain weight (Figure 7): that is, the more stem NSC transferred to the grain from 0–21 DAH, the higher the seed setting rate and grain weight. These results are consistent with those presented in Figure 6.

Figure 7. Relationship between seed setting rate (a), grain weight (b), and the ATM_{NSC} of field rice during 0–21 DAH. The SD1, SD2, and SD3 refer to rice transplanting dates on 20 June, 30 June, and 10 July, respectively.

4. Discussion

Previous studies have shown that the accumulation of NSC increased significantly under elevated $[CO_2]$ [4,29,41]. Our study showed that C_{NSC} and TM_{NSC} in rice stems treated with elevated $[CO_2]$ were higher than the values measured in T0 at the heading stage (Figure 2 and Table 3). This is probably due to the elevated $[CO_2]$ facilitating photosynthetic assimilation and increasing sucrose phosphate synthase and sucrose synthase activities [29]. Photoassimilates are partitioned into sucrose and transferred to vegetative organs [42]. Thus, NSC accumulation was higher under elevated $[CO_2]$. Nevertheless, C_{NSC} and TM_{NSC} did not show a consistent regularity among treatments at the filling and mature stages, possibly because the NSC further translocated and accumulated at the late filling stage.

High fertilizer N in the N3 treatment reduced the C_{NSC} and TM_{NSC} in rice stems at the heading stage (Figure 2), which was consistent with previous studies [18,20]. This was due to enzyme activities related to starch synthesis, such as starch synthase, starch branching enzyme, and adenosine diphosphate–glucose pyrophosphorylase, which all decreased at high N application [18]. High N application led to an increase in plant height, tiller number, and leaf area [32]. The formation of organs, such as leaf blades and stems, require many carbohydrates for cellulose, hemicellulose, and lignin, etc. [43], leading to the decreased storage of NSC in stems and leaves. Yoshida and Ahn [43] also found that the low NSC content in stems was associated with a high N content and a high percentage of leaf blades to stems. When rice plants actively absorb N and produce more leaf blades, photosynthetic products are preferably used for the synthesis of protein and the production of leaf blades. Therefore, the NSC content in stems tended to be lower. Furthermore, carbon skeletons are needed for the conversion of inorganic N absorbed from the soil into organic N. When plants are grown at higher N

fertilizer levels, they require more carbon to meet N metabolism demands [30], which may also be the reason for the lower C_{NSC} in rice stems under high N application.

Low N treatment increased stem NSC translocation to developing grain and its apparent contribution to yield formation [18,20,44]. In our study, the ATM_{NSC} and AC_{NSC} were not prominently different among treatments overall (Table 3), which may be because the NSC accumulated again in the late filling stage (Figure 2 and Table 3). Yoshinaga et al. [45] proved that NSC accumulation was the lowest 20 DAH for indica—Dominant varieties and at 30 DAH for japonica—Dominant varieties. There was a second enrichment and upswing of NSC in rice, which resulted in ATM_{NSC} and AC_{NSC} being negative [46]. Meanwhile, results of different transplanting date treatments identified that the single grain weight of SD1, SD2, and SD3 rice 28 DAH was approximately 85%, 86%, and 75%, respectively, of that at maturity (Figure 8). Consistent with the smaller values of C_{NSC} and the TM_{NSC} of SD3 relative to SD2 and SD1 rice 28 DAH (Figure 3), the percentage of single grain weight 28 DAH to mature grain weight was the lowest in late transplanting rice of SD3 (Figure 8). It is speculated that NSC accumulated again mainly because the grains, as the sink organs, had almost finished grouting, and their ability to absorb photosynthate decreased [47]. Therefore, NSC in stems further accumulated at the end of the filling stage. This result also implied that the grain yield of “Nanjing 9108” was chiefly sink-limited, and improving the sink production efficiency would contribute to increasing its yield potential. It should be noted that our study was carried out within one year. Although we identified the impact of elevated $[CO_2]$ and nitrogen application on NSC accumulation and transference in rice, the contribution of NSC in stem to grain before heading was evaluated only by the difference of NSC accumulation between heading and maturity. Given the accumulation and transference of NSC in plants is a continuous process, dynamically determining the NSC concentration in crop stems will be more accurate in assessing the contribution of NSC stored in stems before heading to the grain yield.

Figure 8. Dynamics of single grain weight under different transplanting date treatments. The SD1, SD2, and SD3 refer to rice transplanting date on 20 June, 30 June, and 10 July, respectively. Same lowercase letters indicate no significant effects ($p > 0.05$) of transplanting dates on the single grain weight of rice. DAH is days after heading.

It has been proven that the remobilization of NSC stored in stems before heading plays an important role in rice grain filling [18,20,28,48,49]. Li et al. [50] showed that the percentage of filled grains was notably and positively correlated with the TM_{NSC} in stems and NSC per spikelet at anthesis in rice, and the enhanced NSC remobilization during grain filling could contribute not only to an increase in filling efficiency, but also to a higher harvest index. Nagata et al. [51] observed that NSC stored in stems before heading tended to increase the seed setting rate when dry matter production

after heading was insufficient, and the period that had the greatest impact on rice grain filling was 10–20 DAH. Experimental studies on rice, barley, and millet have consistently shown that the ATM_{NSC} during the filling stage was significantly positively correlated with grain yield and the 1000-grain weight [20,34,52]. In this study, the seed setting rate of rice at the filling stage was positively related to ATM_{NSC} (Figure 6), and the field rice during the 0–21 DAH period also showed that the larger the ATM_{NSC} , the higher the seed setting rate and yield (Figure 7). This confirms the importance of stem NSC in grain filling and yield enhancement.

5. Conclusions

The C_{NSC} and TM_{NSC} under treatment with elevated $[CO_2]$ were higher than those in the T0 treatment, and of which the N3 treatment was lower than that of N1 and N2 at the heading stage. There was no significant difference in AC_{NSC} among treatments, and the stem NSC accumulated markedly during the late filling stage, implying that the grain yield of “Nanjing 9108” was predominantly sink-limited. The larger the ATM_{NSC} , the more NSC transferred from stems to grains, and the higher the seed setting rate at the filling stage, but there was no significant relationship between grain yield and ATM_{NSC} . This research has no special requirements for the environment and can be carried out in a broader area where japonica rice of “Nanjing 9108” is planted. Based on our results, we recommend that future studies focus on increasing the sink capacity and promoting the translocation of stem NSC to grains through genetic improvement, breeding, or the development of field management methods to attain higher and stable rice grain yield in the context of climate change.

Author Contributions: Conceptualization, P.C., W.S., and Y.H.; methodology, P.C., W.S., and Y.H.; data attainment, P.C., J.Y., K.Y., C.L., Y.W., L.Y., Z.H.; writing—Original draft preparation, P.C.; All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China, grant number 41530533.

Acknowledgments: We thank Tianhang Create technology Co. Ltd. for providing technical support for the CO_2 concentration control system, and Cailin Wang of Jiangsu Academy of Agricultural Sciences for providing rice seeds.

Conflicts of Interest: The authors declare no conflict of interest.

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