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Synergistic nitrogen fertiliser effects on nitrogen metabolism of wheat in saline-alkaline land

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Abstract: In this study, a synergist made of itaconic acid, maleic acid, acrylic acid and other active ingredients polymerised was sprayed on the surface of nitrogen (N) fertiliser particles to make synergistic nitrogen fertilisers (SNF). To explore the effect of SNF on N metabolism of wheat in saline alkaline land, five treatments were set up: CK – ordinary N fertiliser (299.86 kg N/ha); T1 – SNF (299.86 kg N/ha); T2 – SNF (239.89 kg N/ha); T3 – SNF (179.92 kg N/ha); T4 – SNF (119.94 kg N/ha). The aboveground dry weight of wheat, the photosynthetic characteristics of wheat flag leaves, the activity of the N metabolism enzyme of wheat flag leaves, the expression of N transporter-related genes in wheat roots, and the N accumulation and transport of plants were determined. The results showed that the T1 treatment performed the best. During the two years, the N translocation from stems and leaves to spikes of plants at maturity in T1 was 33.18–45.55% higher than that of CK. The N content of wheat spikes was 12.01–12.66% higher than that of CK. The activities of nitrate reductase, glutamine synthetase, glutamate synthetase and the expression of nitrate transporter gene *TaNRT1.1* and ammonium transporter gene *TaAMT1.1* were significantly higher than that of CK. The aboveground dry weight of wheat and photosynthetic characteristics of flag leaves were significantly higher than those of CK in T1, whereas the intercellular CO_2 concentration was significantly lower than that of CK. The application of SNF positively affected N accumulation and transport in wheat, wheat yield, and fertiliser utilisation, as well as reduced N loss in saline alkaline land.

Keywords: saline-alkali stress; nitrogen use efficiency; gene expression; enzyme activity; photosynthesis

Wheat (*Triticum aestivum* L.) is one of the most important food crops in the world, accounting for 30% of global grain production. It is rich in several nutrients, thus considered an important source of energy and protein for about one-third of the population around the globe; more than one-third of the world's population depends on wheat products as a staple food (Zorb et al. 2018, Li et al. 2019, Schittenhelm et al. 2020). Due to the trend of increasing world population and decreasing arable land year by year (Foley et al. 2011, Tilman et al. 2011), food security and safety may be a big challenge by the year 2050 (Ray et al. 2013). Therefore, increasing crop production to cope with "food security" is very urgent. In recent years, with the acceleration of industrialisation and urbanisation, the damage to the ecological environment has intensified, and crop production is compromising due to several biotic and abiotic stress factors. Soil salinisation is one of the most serious adversity conditions restricting crop growth and affecting crop yield. Saline soils contain more saline components, higher soil conductivity and soil pH, and saline factors such as chlorides and bicarbonates in saline-alkaline soils are responsible for

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soil hardening and soil infertility (Li et al. 2023). So far, more than 3% of the world's soil is affected by salinisation, and it continues to increase at a rate of two million hectares per year (Singh 2018).

Nitrogen (N) plays an important role in plant growth, and plants mainly take up N from the soil as a mineral nutrient and in the form of ammonium (NH_4^+) and nitrate (NO_3^-). Nitrate is transported primarily through members of the NRT family of NO_3^- transporter proteins. The most widely studied NRT gene is *NRT1.1*, which is induced by NO_3^- and encodes a dual-affinity transporter with both high-affinity and low-affinity system activity (Parker and Newstead 2014). Similar to the NRT family, the ammonia transport protein (AMT) genes are divided into *AMT1* and *AMT2* based on affinity level, whereas due to the low NH_4^+ concentration in the soil, the uptake of NH_4^+ by the plant mainly uses the high-affinity transport system (Bajgain et al. 2018). In the cytoplasm, nitrate is catalysed by nitrate reductase (NR) to nitrite, which is transferred to the chloroplasts and reduced to ammonium by nitrite reductase (NiR), and the resulting ammonium is supplied to the plant through the assimilation of glutamine synthetase (GS) and glutamate synthetase (GOGAT), which uses energy released from ATP catabolism to convert inorganic N to organic N such as glutamate and glutamine for plant utilisation (Suzuki and Knaff 2005). However, high soil salinity significantly affects N transport transformation, uptake, and utilisation in agricultural fields. Many studies have reported that increasing salinisation may have adverse effects on N use efficiency due to higher N losses through volatilisation and denitrification (Li et al. 2020). Salt deposition in the rhizosphere due to increasing salinisation causes osmotic stress to restrict water extraction by plant roots. It leads to lower N absorption and assimilation. Zhang et al. (2020) found that salt stress significantly reduced wheat chlorophyll content, photosynthetic capacity and N uptake, whereas root N metabolism would be significantly enhanced due to increased GS activity. Guo et al. (2015) found that alkali stress significantly suppressed the photosynthetic rate of wheat, resulting in reduced sugar production and limited N metabolism.

In agricultural production, the application of N fertiliser can increase the photosynthetic rate and translocation of photosynthates, improve crop yield and quality (Xu et al. 2021), and enhance plant resistance to environmental stresses such as drought stress and salt stress (Li et al. 2020). Only about 42–47% of the N added to farmland globally is absorbed and transformed into crop products (Mueller et al. 2017). In China, the N fertiliser application rate is 305 kg/ha, which is more than four times the global average, but the N utilisation efficiency is only 25% (Zhang et al. 2015). In recent years, the application of slow-release fertilisers has been regarded as an effective measure to reduce nutrient losses, improve fertiliser utilisation (FU), solve leaching problems and reduce the amount of fertiliser applied (Ramli 2019). Slow/controlled release fertiliser can gradually release nutrients fertilisers to crops at a certain rate, which can meet the fertiliser requirements of crops throughout the growth period, improve the absorption efficiency of nutrients by plants, and reduce the risk of excessive fertilisation. At the same time, slow/controlled release fertilisers are often provided with physical barriers on the surface of fertilisers, which is used to protect fertilisers from degradation by soil microorganisms and loss by leaching (Bansiwal et al. 2006, Davidson and Gu 2012). It has the advantages of efficiency enhancement, long-lasting effect and stable effect. However, the slow-release fertiliser coating process is complex, and the required materials are expensive. Therefore, there is a need to select suitable materials and methods to prepare fertilisers with slow-release nutrient effects. Chen et al. (2023) used a hydrogel formed by the polymerisation of IA, MA, AA and potassium persulfate (KPS) to make a synergistic fertiliser to improve its slow-release performance and increase the efficiency of the fertiliser. Li et al. (2023) polymerised taconic acid (IA), maleic acid (MA), acrylic acid (AA), and KPS into synergists and wrapped them around the surface of phosphorus fertiliser particles to form synergistic phosphorus fertilisers, which increased the content of soil alkaline dissolved N, effective phosphorus, and effective potassium (K), and had a positive effect on soil microbial diversity. Currently, there are fewer studies on applying synergists to N fertilisers. Therefore, in this experiment, a polymer hydrogel coating made of IA, MA, AA and other active ingredients was sprayed on the surface of ordinary urea particles to make SNF with particle size similar to that of ordinary fertilisers and its effect on N metabolism of wheat in saline-alkaline land was explored by setting up different gradients of SNF application, with a view to providing theoretical support and practical basis for its application in wheat production.

MATERIAL AND METHODS

Experimental site and environmental conditions. A two-year field experiment was conducted from

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Table 1. Basic soil fertility level

Year	Alkaline hydrolysis nitrogen	Available phosphorus	Available potassium	Organic carbon	Soil pH	Calcium	Magnesium	Soil electrical conductivity
	(mg/kg)			(g/kg)		(g/kg)		(μS/cm)
2020–2021	60.04	16.93	231.67	7.71	7.92	3.12	1.27	721
2021–2022	65.13	18.70	226.37	7.62	7.84	3.29	1.29	703

October 2020 to June 2021 and from October 2021 to June 2022 in Liutuan Town, Weifang City, Shandong Province, China (37°00'27.24"N, 119°22'15.79"E). The experimental field belongs to the temperate monsoon climate. The average temperature during wheat growth in 2020–2021 and 2021–2022 was 10.8 °C and 11.5 °C, and the total precipitation during wheat growth in 2020–2021 and 2021–2022 was 130.3 mm and 297.4 mm, respectively. Before wheat sowing, the basic soil fertility level of the test plot is shown in Table 1. The soil salt content is 0.3%, which is mildly saline. The test plot is close to the seaside area, and the compounds that inhibit plant growth are mainly calcium (Ca), K, sodium (Na) and magnesium (Mg) chlorides. The monthly precipitation and average monthly temperature during the test period are shown in Figure 1.

Experimental materials. The wheat cultivar selected for the experiment was Taimai 198, which is a winter, dwarf and medium-multi-spike cultivar with good cold resistance, strong adaptability and high yield potential. Urea was used as N fertiliser, triple superphosphate as phosphate (P) fertiliser, and K sulfate as K fertiliser. IA, MA and AA were purchased from Aladdin Biochemical Technology Co., Ltd (Shanghai, China).

Experimental design. A randomised block design was used in the experiment. Five different N fertiliser treatments were set up, each treatment was repeated 3 times, and a total of 15 experimental plots were made. The area of each plot was 15 m^2 (3 m × 5 m), with an interval of 1 m between plots. The amount of fertiliser applied to each treatment is detailed in Table 2. Both P and K fertilisers were applied at 166.59 kg/ha and 58.3 kg/ha for all treatments. The sowing method is mechanical strip sowing. The first-year trial was sown on October 16, 2020, at a seeding rate of 187.5 kg/ha and harvested on June 14, 2021. The second-year trial was sown on October 11, 2021, at a rate of 187.5 kg/ha and harvested on June 12, 2022. Before sowing, P fertiliser, K fertiliser, ordinary nitrogen fertiliser (ONF) and synergistic nitrogen fertilisers (SNF) were applied as base fertiliser, and no topdressing was applied later. During the growth period of wheat, only natural precipitation is relied on.

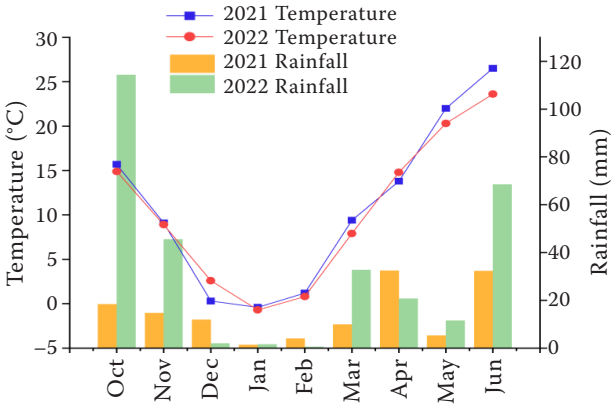

Figure 1. Average temperature and precipitation of different months during the experiment period

Measurement items and methods

Soil nutrient determination. Wheat 0–20 cm soil layer samples were collected and air-dried at room temperature under shaded conditions. Air-dried soil samples were sieved through a 1 mm sieve. Soil organic carbon of air-dried soil samples was determined by potassium dichromate-sulphuric acid digestion method (Zhang and Gong 2012), soil alkaline N

Table 2. Fertilisation amount of each treatment

Treatment	Treatment content	Amount of nitrogen fertiliser application (kg/ha)
CK	ONF	299.86
T1	SNF	299.86
T2	SNF	239.89
T3	SNF	179.92
T4	SNF	119.94

ONF – ordinary nitrogen fertiliser; SNF – synergistic nitrogen fertiliser

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was determined by the alkaline diffusion method (Bao 2000), the determination of soil pH refers to the potentiometric method (water/soil = 5:1, using a PHS-3C pH meter, Shanghai, China), determination of available P in soil by molybdenum-antimony colourimetric method (Olsen 1954), determination of soil available K content by ammonium acetate leaching method (Merwin and Peech 1951). Accurately weigh 0.25 g of soil sample in a plastic centrifuge tube, moisten with a small amount of water, add 1.0 mL of hydrochloric acid, 1.0 mL of nitric acid, and 2.0 mL of hydrofluoric acid and shake well, tighten the lid and then loosen it by 1/6th of the way with a backswing and let it stand overnight. The centrifuge tube with the sample was put into a constant temperature graphite digestion furnace, heated at reflux for 1 h, removed and cooled, and then fixed to 25 mL with deionised water and shaken well, and then centrifuged and examined for the content of Ca and Mg by Inductively Coupled Plasma Emission Spectroscopy (ICP-OES, Waltham, USA) of PerkinElmer Instruments Co.

The aboveground dry weight of wheat. Samples were taken at the anthesis (0 days after anthesis), 10, 20, and 30 days after anthesis, and wheat maturity, and 10 wheat single stems with relatively consistent growth were randomly cut from each plot. The single stem was divided into three parts: stem, leaf and ear, and dried in an oven (dry at 105 °C for 20 min and then dry at 75 °C until constant weight), and weighed the dry weight of each part after cooling to room temperature.

The aboveground dry weight of wheat population (kg/ha) = an effective number of spikes per plot × aboveground dry weight of a single wheat stem. (The effective number of spikes is the number of spikes on each wheat plant that are actually involved in grain formation.)

Photosynthetic properties of wheat flag leaf. Samples were taken at the anthesis (0 days after anthesis), 7, 14, 21, and 28 days after anthesis of wheat, respectively. From 9 to 11 a.m., 10 wheat flag leaves were randomly selected from each plot, and the net photosynthetic rate, intercellular CO_2 concentration, and stomatal conductance (SC) were determined using the CIRAS-3 portable open photosynthesis meter (Hansatech, Amesbury, USA), and the chlorophyll content was determined using the SPAD-502 (Tokyo, Japan) portable chlorophyll meter, and the average value was taken from multiple measurements.

Activities of enzymes related to N metabolism in flag leaf of wheat. The NR activity, GS activity and GOGAT activity were measured. Samples were taken at the anthesis (0 days after anthesis), 7, 14, 21, and 28 days after anthesis of wheat, respectively. In each plot, six wheat plants with consistent growth were selected to cut flag leaves, stored in self-sealing bags, wrapped in tin paper, quickly placed in liquid N, and transferred indoors to a refrigerator at –80 °C for testing. The activity of N metabolism enzymes was determined according to the method used by Effah et al. (2023).

Expression of N transporter related genes in wheat roots. Samples were taken at the anthesis (0 days after anthesis), 7, 14, 21, and 28 days after anthesis of wheat, respectively. In each plot, 3 wheat plants with the same growth were selected and their roots were taken. They were stored in self-sealing bags, wrapped in tin paper, quickly placed in liquid N, and then transferred indoors to a –80 °C refrigerator for testing. The roots were quickly ground in liquid N and RNA was extracted using the SteadyPure Plant RNA Extraction Kit. The concentration and purity of extracted RNA were detected by NanoDrop RNA quality detector. The integrity of extracted RNA was determined by agarose gel electrophoresis. Evo M-MLV RT Mix Kit was used to synthesize cDNA by reverse transcription. SYBR Green Premix Pro Taq HS qPCR Kit was used for fluorescence quantitative PCR reaction. The kits were purchased from Accurate Biotechnology Co., Ltd. (Hunan, China).

The name of assay gene and the corresponding primer sequence were determined with reference to Li et al. (2019), and the ADP-RF gene (Paolacci et al. 2009) was used as the internal reference. The specific sequence is shown in Table 3.

Table 3. Sequences of all primers used in quantitative real-time PCR

Gene		Sequence 5'–3'
Ammonium transporter gene *TaAMT1.1*	F	ACAGCTTCTTCCTCTTCC
	R	CCGAGTAGATGAGGTAGG
Nitrate transporter gene *TaNRT1.1*	F	ATGCCAGGTTGTCATTGC
	R	CCGAGTCCAGTTGTATGC
Reference gene *ADP-RF*	F	GCTCTCCAACAACATTGCCAAC
	R	GCTTCTGCCTGTCACATACGC

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Plant N content. Samples were taken at the anthesis (0 days after anthesis), 10, 20, and 30 days after anthesis, and wheat maturity and dried ground into powder using a plant universal pulveriser and kept for measurement. The total N content of the plants was determined using a Kjeldahl N meter.

Plant N accumulation and transport. Calculated using the data measured in aboveground dry weight and plant N content of wheat.

N accumulation in each part = dry weight of each part × N content of each part.

N accumulation in the aboveground part of the plant = sum of N accumulation in each part of the aboveground part.

N translocation amount by plant stems and leaves = N accumulation by plant stems and leaves at anthesis – N accumulation by plant stems and leaves at maturity.

N translocation rate = N translocation amount from plant stems and leaves/N accumulation from plant stems and leaves at anthesis (Pei et al. 2022).

Statistical analysis. Microsoft Excel 2021 (Redmond, USA) and SPSS Statistics 26 software (IBM, New York, USA) were used for data processing, significance tests and analysis of variance, and multiple comparisons were performed using Duncan's test when $P < 0.05$, and the marked letter method was used to indicate the differences between treatments. Graphing of all test data was performed by Origin 2022 software (OriginLab, Inc., Massachusetts, USA) for Windows systems.

RESULTS

Effect of SNF on post-flowering growth of wheat in saline soils

Effect of SNF on aboveground dry weight of wheat. As shown in Figure 2, the aboveground dry weight of wheat plants in all treatments showed an increasing trend with the growth and development of wheat. In 2021, the aboveground plant dry weight of wheat at maturity T1 was significantly higher than that of all other treatments, while T1, T2, and T3 at maturity were increased by 8.29, 10.97, and 1.74%, respectively, compared to CK, and T4 was decreased by 4.04%. In 2022, the dry weight of wheat plants above ground was generally higher in all treatments than in 2021, with less difference among treatments. Aboveground dry weight of wheat at anthesis showed T1 > T2 > T3 > CK > T4 in descending order. In the comparisons, the differences between neighbouring treatments were not significant, and the treatments spaced apart from each other were significantly different. At maturity, T1, T2, and T3 were 18.14, 10.19, and 2.72% higher than CK, respectively, while T4 was 3.95% lower than CK. In conclusion, under the same fertilisation amount, the SNF has obvious promotion effect on wheat's aboveground dry matter accumulation.

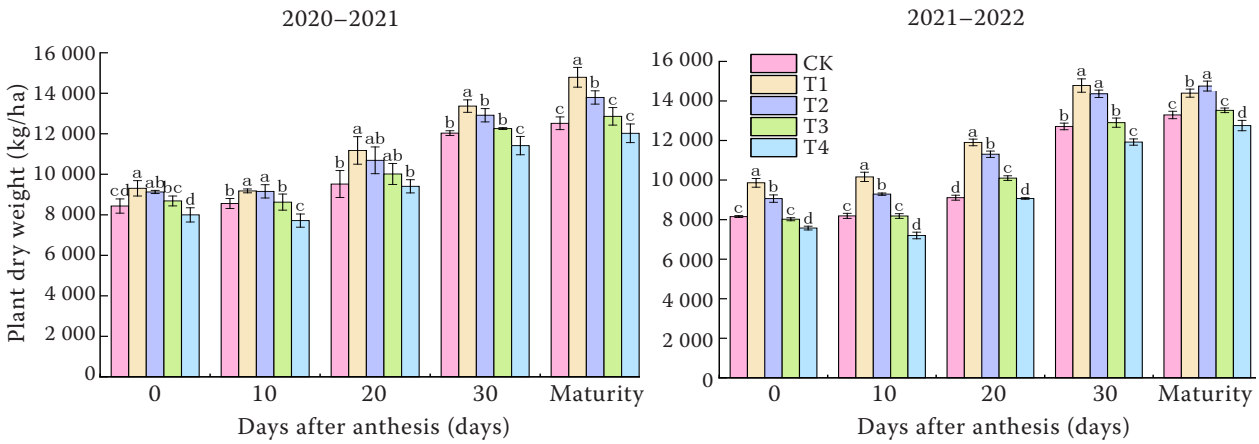

Figure 2. Changes of aboveground dry weight of wheat plants after anthesis. The type of mean comparison test was Duncan's new multiple-range method. The vertical bar represents the standard error, and the different letters above the error line represent the significant difference in the mean values of different treatments of the same measurement item ($P < 0.05$). CK – ordinary nitrogen fertiliser (299.86 kg N/ha); T1 – synergistic nitrogen fertilisers (SNF) (299.86 kg N/ha); T2 – SNF (239.89 kg N/ha); T3 – SNF (179.92 kg N/ha); T4 – SNF (119.94 kg N/ha)

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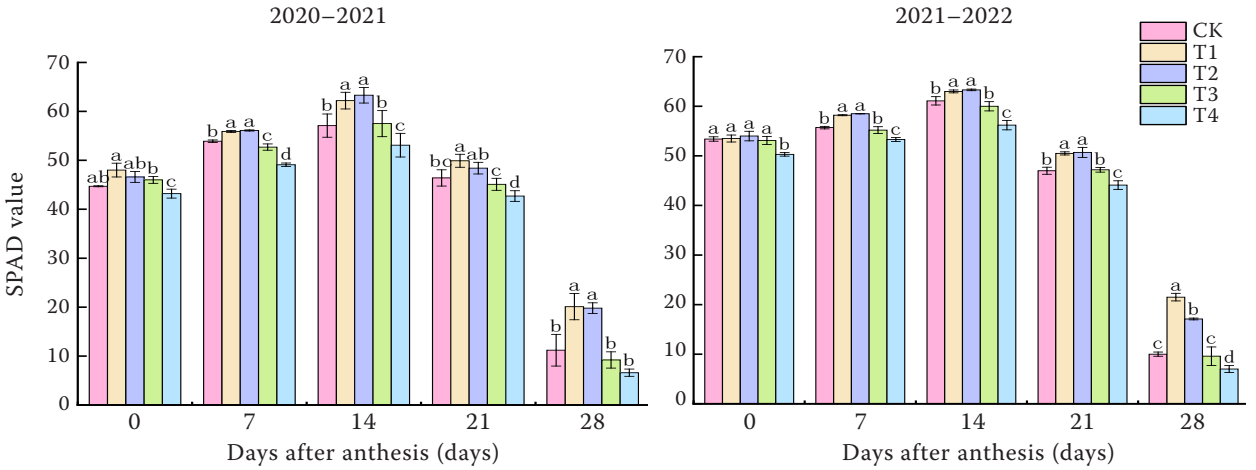

Figure 3. SPAD value of flag leaf of wheat after anthesis. The type of mean comparison test was Duncan's new multiple-range method. The vertical bar represents the standard error, and the different letters above the error line represent the significant difference in the mean values of different treatments of the same measurement item ($P < 0.05$). CK – ordinary nitrogen fertiliser (299.86 kg N/ha); T1 – synergistic nitrogen fertilisers (SNF) (299.86 kg N/ha); T2 – SNF (239.89 kg N/ha); T3 – SNF (179.92 kg N/ha); T4 – SNF (119.94 kg N/ha)

Effect of SNF on the photosynthetic properties of wheat flag leaves

Effect of SNF on chlorophyll content of wheat flag leaves. As shown in Figure 3, the SPAD values of wheat flag leaves of each treatment showed a trend of increasing and then decreasing with the advancement of the reproductive process, which gradually increased from 0 to 14 days after anthesis, but the magnitude of the increase was small, and then began to decrease after reaching the highest value at 14 days after anthesis, and then decreased abruptly at 21–28 days after anthesis, and the trend was basically the same for the two years, but the SPAD values of the 2022 were at the higher level. The SPAD values of wheat flag leaves of each treatment basically showed T1 > T2 > CK > T3 > T4, but there was no significant difference between T1 and T2 and between CK and T3. In 2021, SPAD values of wheat flag leaves of T1 and T2 were 79.17% and 76.77% higher than CK at 28

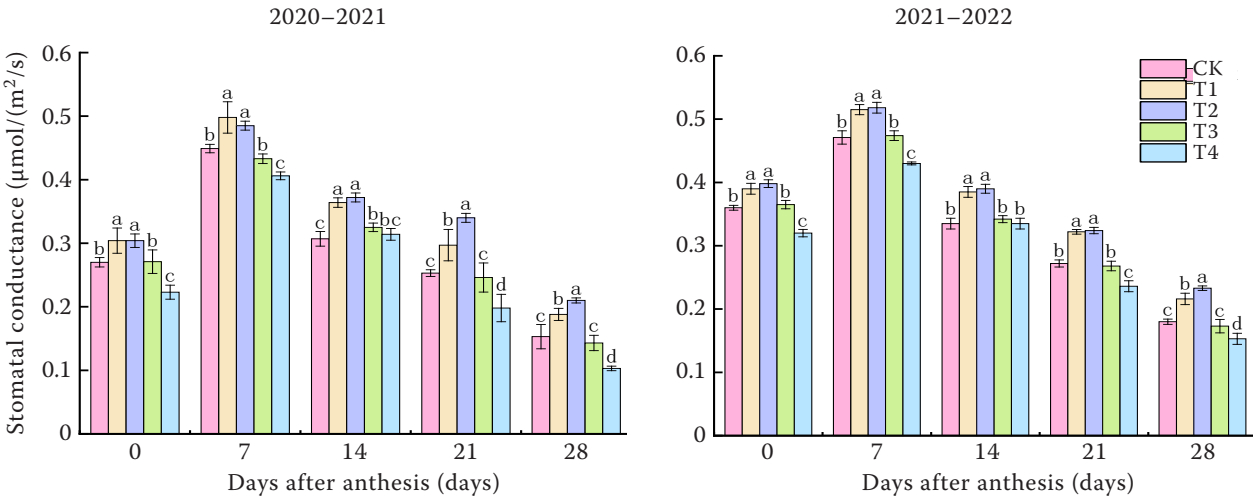

Figure 4. Stomatal conductance of flag leaf of wheat after anthesis. The type of mean comparison test was Duncan's new multiple-range method. The vertical bar represents the standard error, and the different letters above the error line represent the significant difference in the mean values of different treatments of the same measurement item ($P < 0.05$). CK – ordinary nitrogen fertiliser (299.86 kg N/ha); T1 – synergistic nitrogen fertilisers (SNF) (299.86 kg N/ha); T2 – SNF (239.89 kg N/ha); T3 – SNF (179.92 kg N/ha); T4 – SNF (119.94 kg N/ha)

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days after anthesis, respectively. In 2022, T1 and T2 were 1.15 and 0.71 times higher than CK at 28 days after anthesis, respectively. The above results indicated that the application of SNF could keep the SPAD value of wheat flag leaf at a high level after anthesis and significantly reduce the decrease of SPAD value at the later stage.

Effect of SNF on stomatal conductance of wheat flag leaves. As shown in Figure 4, the SC of wheat flag leaves of all treatments showed a trend of increasing and then decreasing with the increase of days after anthesis, which increased significantly from 0 to 7 days after anthesis and then decreased gradually from 7 to 28 days after anthesis, and in 2022, the SC was large and decreased at a lower rate. The size order of SC of each treatment between two years basically showed that T2 > T1 > T3 > CK > T4, but T1 and T2 treatments, SC of wheat flag leaves in the early stage was not significantly different, and in the late stage, T2 was significantly higher than that of T1; there was no significant difference between CK and T3 treatments. In 2021, the SC of T1 and T2 flag leaves increased by 10.84% and 8.02%, respectively, while those of T3 and T4 decreased by 9.65% and 3.56%, respectively. In 2022, the SC of flag leaves of T1, T2 and T3 increased by 9.34, 10.05 and 0.57%, respectively, compared with CK on the 7 days after anthesis, while that of T4 decreased by 8.78%. The above results showed that the application of SNF increased the SC of the flag leaves after anthesis, and the effect could be maintained after a 20% reduction.

Effect of SNF on intercellular CO_2 concentration in wheat flag leaves. As shown in Figure 5, the changes in the intercellular CO_2 concentration in the flag leaf of wheat after anthesis were basically the same in all treatments, all of which increased gradually with the advancement of the reproductive process, and the difference in the intercellular CO_2 concentration in the flag leaf of wheat in the two years was not significant. The intercellular CO_2 concentration in wheat flag leaves of each treatment showed from low to high T2 < T1 < T3 < CK < T4, and the variability among treatments decreased gradually with the increase of days after flowering. In 2021, the intercellular CO_2 concentration in wheat flag leaves at anthesis was reduced by 15.12% and 16.39% in T1 and T2, respectively, compared with CK, and increased by 1.27% and 16.58% in T3 and T4, respectively, compared with CK. In 2022, the intercellular CO_2 concentration in wheat flag leaves at anthesis was reduced by 15.08% and 17.5% at T1 and T2, respectively, compared with CK, and increased by 2.24% and 14.52% at T3 and T4, respectively, compared with CK. The above results showed that the intercellular CO_2 utilisation of wheat flag leaf was higher after the application of SNF.

Effect of SNF on the transpiration rate of wheat flag leaves. As shown in Figure 6A, the TR of post-

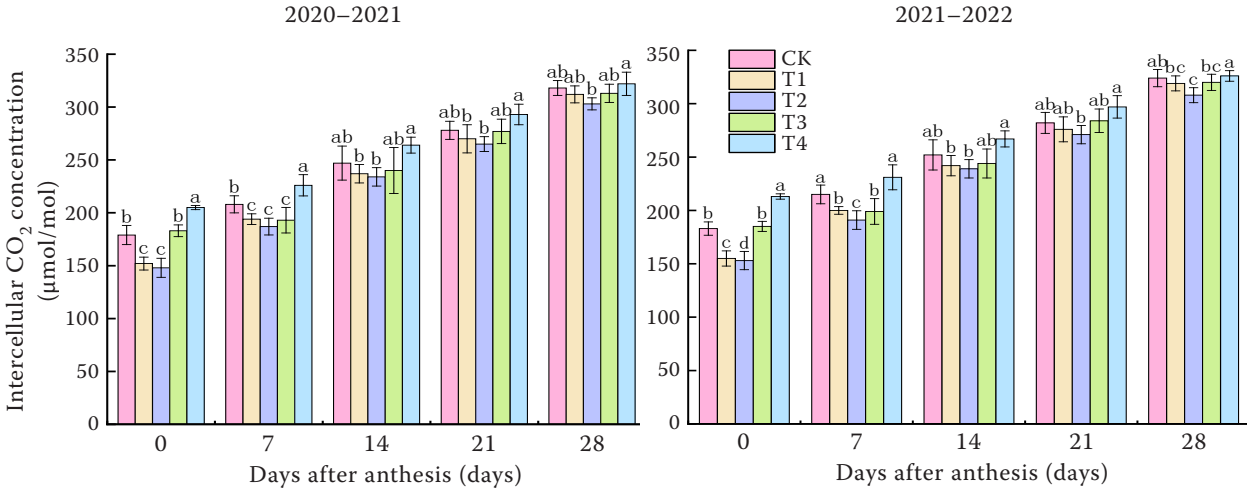

Figure 5. Intercellular CO_2 concentration in flag leaves of wheat after anthesis. The type of mean comparison test was Duncan's new multiple range method. The vertical bar represents the standard error, and the different letters above the error line represent the significant difference in the mean values of different treatments of the same measurement item ($P < 0.05$). CK – ordinary nitrogen fertiliser (299.86 kg N/ha); T1 – synergistic nitrogen fertilisers (SNF) (299.86 kg N/ha); T2 – SNF (239.89 kg N/ha); T3 – SNF (179.92 kg N/ha); T4 – SNF (119.94 kg N/ha)

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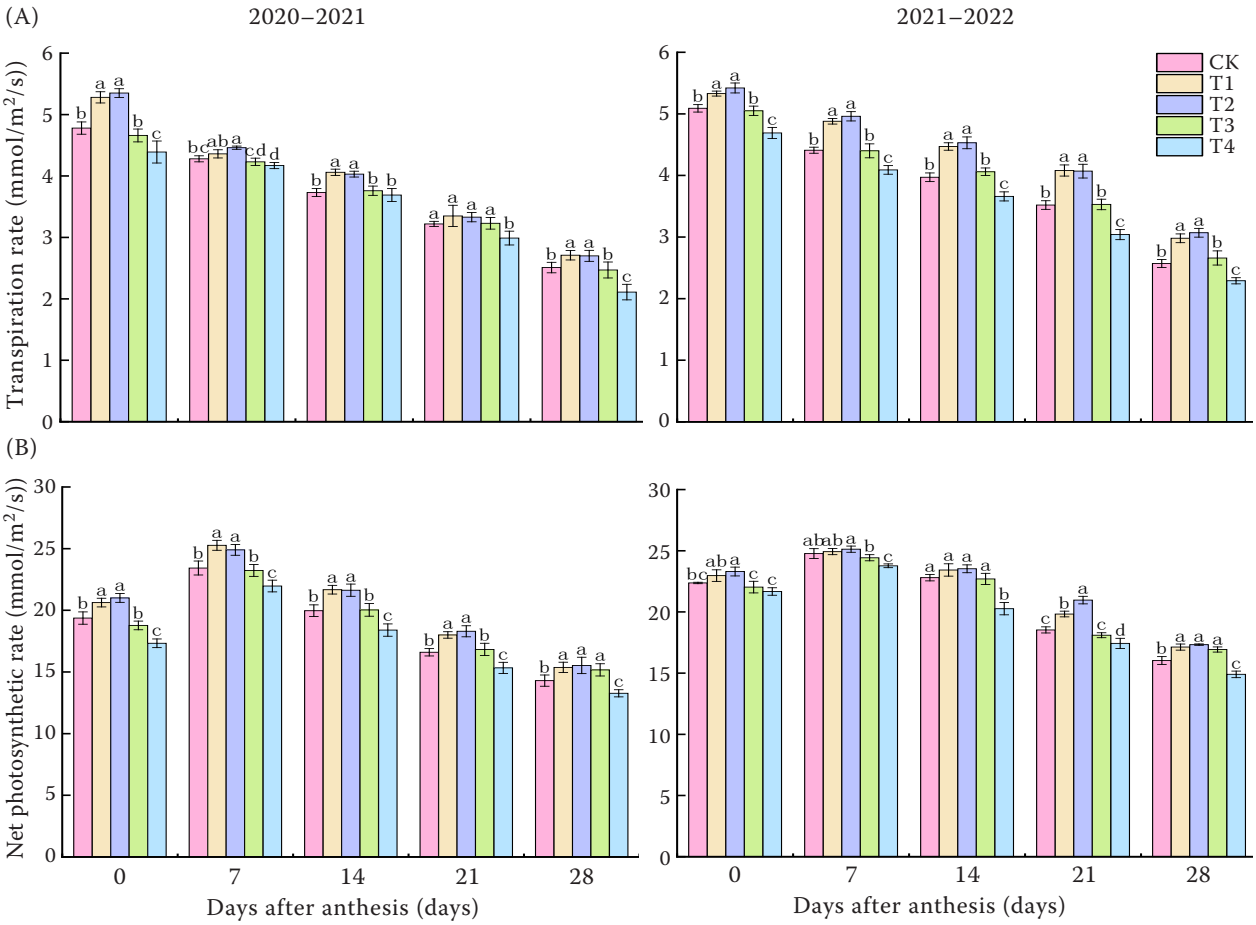

Figure 6. (A) Transpiration rate and (B) net photosynthetic rate of flag leaf of wheat after anthesis. The type of mean comparison test was Duncan's new multiple range method. The vertical bar represents the standard error, and the different letters above the error line represent the significant difference in the mean values of different treatments of the same measurement item ($P < 0.05$). CK – ordinary nitrogen fertiliser (299.86 kg N/ha); T1 – synergistic nitrogen fertilisers (SNF) (299.86 kg N/ha); T2 – SNF (239.89 kg N/ha); T3 – SNF (179.92 kg N/ha); T4 – SNF (119.94 kg N/ha)

flowering flag leaves of wheat varied consistently across treatments, all gradually decreasing as fertility progressed. In 2022, the flag leaf TR of wheat after anthesis was at a high level and differed significantly among treatments. The TR of wheat flag leaves after anthesis in all treatments showed T1 > T2 > T3 > CK > T4 from high to low, but there was no significant difference between T1 and T2 and between T3 and CK. In 2021, from 0 to 28 days after anthesis, the TR of CK, T1, T2, T3, and T4 were reduced by 47.38, 48.7, 49.5, 46.96, and 51.97, respectively. In 2022, from 0–28 days after anthesis, TR were reduced by 49.41, 44.06, 43.45, 47.23, and 51.21% in CK, T1, T2, T3, and T4, respectively.

Effect of SNF on net photosynthetic rate of wheat flag leaves. As shown in Figure 6B, the NPR of the flag leaf of wheat after anthesis in all treatments showed an increasing and then decreasing trend with the progression of reproductive process. In 2022, the NPR of flag leaves of wheat after anthesis was at a high level. The NPR of wheat flag leaves after anthesis in each treatment showed T2 > T1 > T3 > CK > T4 from high to low, but there was no significant difference between T1 and T2, and no significant difference between CK and T3. In 2021, from 0 to 28 days after anthesis, the NPR of CK, T1, T2, T3, and T4 were reduced by 26.16, 25.52, 26.03, 19.18, and 23.46%, respectively. In 2022, the NPR of CK, T1, T2, T3, and T4 were reduced by 28.32, 25.4, 25.61, 23.15, and 31.23%, respectively, from 0–28 days after anthesis. In conclusion, the application of SNF significantly increased the NPR of flag leaves of wheat after the anthesis and narrowed the decrease in the later stage.

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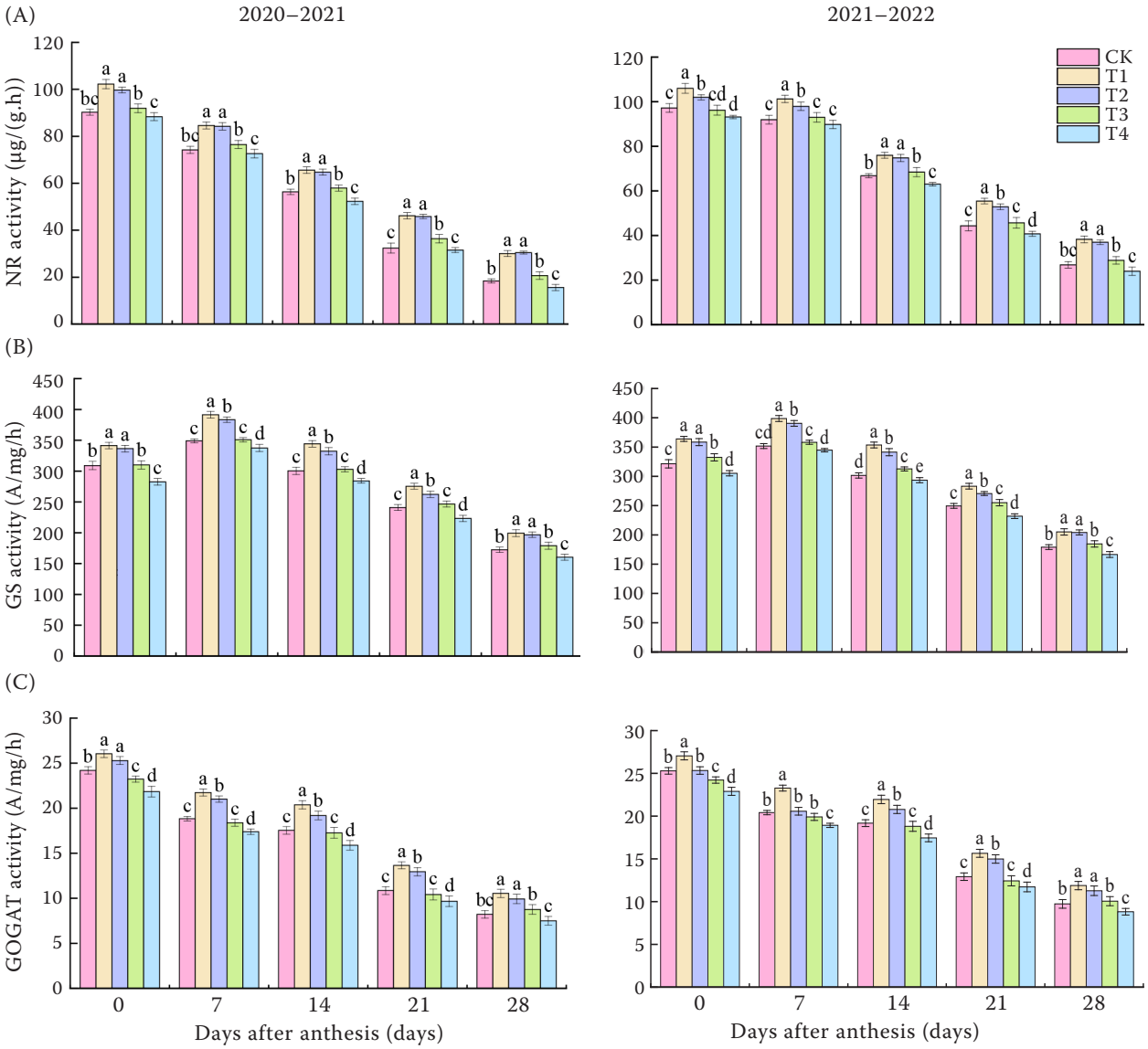

Figure 7. (A) Nitrate reductase (NR); (B) glutamine synthetase (GS) and (C) glutamate synthetase (GOGAT) activity in flag leaf of wheat after anthesis. The type of mean comparison test was Duncan's new multiple-range method. The vertical bar represents the standard error, and the different letters above the error line represent the significant difference in the mean values of different treatments of the same measurement item ($P < 0.05$). CK – ordinary nitrogen fertiliser (299.86 kg N/ha); T1 – synergistic nitrogen fertilisers (SNF) (299.86 kg N/ha); T2 – SNF (239.89 kg N/ha); T3 – SNF (179.92 kg N/ha); T4 – SNF (119.94 kg N/ha)

Effects of SNF on N metabolism-related enzymes in flag leaf wheat

Effect of SNF on the activity of NR in flag leaf of wheat. As shown in Figure 7A, the NR activity of flag leaves after anthesis showed a decreasing trend in all treatments. In 2021, the NR activity of flag leaves increased slightly from anthesis to 7 days after anthesis, decreasing continuously. In 2022, it decreased continuously since the anthesis, but the overall activity was higher than the previous season's. In 2021, the basic performance of each treatment was T1 > T2 > T3 > CK > T4. Among them, the difference between T1 and T2 was not significant, and the difference between T3 and CK was not significant, but T1 and T2 were significantly higher than T3 and CK. In 2022, T1 was significantly higher than T2 for part of the time, and CK was significantly higher than T4 for most of the time, but there was no significant difference between the two in the end. In 2021, the NR activities of CK, T1, T2, T3 and T4 wheat flag leaves decreased by 75.17, 64.48, 63.83, 72.95 and

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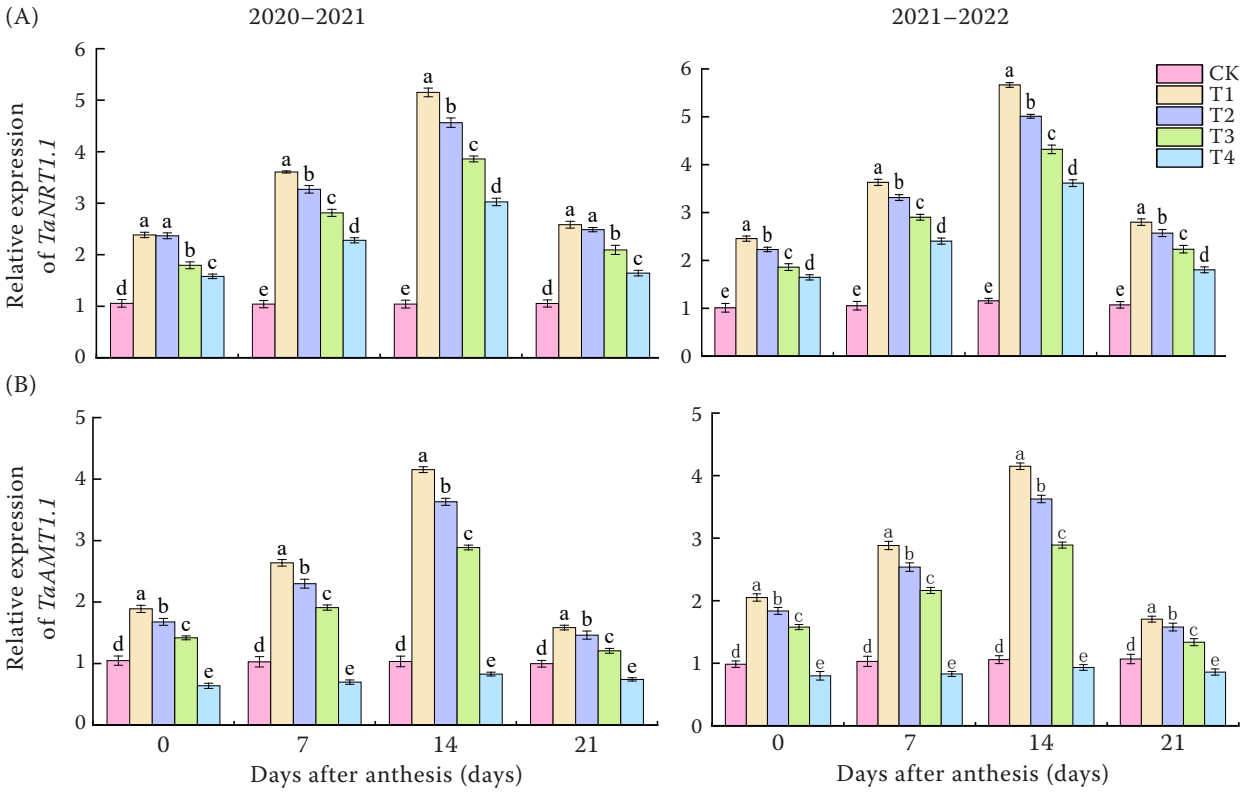

Figure 8. Expression of (A) nitrate transporter gene *TaNRT1.1* and (B) ammonium transporter gene *TaAMT1.1* in wheat after anthesis. The type of mean comparison test was Duncan's new multiple range method. The vertical bar represents the standard error, and the different letters above the error line represent the significant difference in the mean values of different treatments of the same measurement item ($P < 0.05$). CK – ordinary nitrogen fertiliser (299.86 kg N/ha); T1 – synergistic nitrogen fertilisers (SNF) (299.86 kg N/ha); T2 – SNF (239.89 kg N/ha); T3 – SNF (179.92 kg N/ha); T4 – SNF (119.94 kg N/ha)

78.50% respectively at 0–28 days after anthesis. In 2022, it decreased by 72.36, 63.89, 63.72, 70.02 and 74.25%, respectively. It can be seen that compared with the application of ordinary urea, the application of SNF can significantly increase the NR activity of flag leaf after anthesis, and make the reduction less.

Effect of SNF on GS activity in flag leaf of wheat. As shown in Figure 7B, the GS activity in flag leaves of wheat after anthesis showed a trend of increasing first and then decreasing with the advancement of the growth process. It increased slightly from 0 to 7 days after anthesis, and then decreased gradually. The GS activity was at a high level in 2022. In 2021, the treatments at 0 days after anthesis and 28 days after anthesis showed T1 > T2 > T3 > CK > T4, with no significant difference between T1 and T2, and no significant difference between T3 and CK. In 2022, T3 was significantly higher than CK at 0 days and 14 days after anthesis. In 2021, the GS activity of flag leaves of CK, T1, T2, T3 and T4 wheat decreased by 44.21, 41.60, 41.50, 42.25 and 43.25%, respectively, from 0 to 28 days after anthesis. In 2022, they decreased by 44.28, 43.65, 43.04, 44.50 and 45.44%, respectively. It can be seen that the application of SNF can significantly increase the activity of GS in flag leaves after anthesis and make the reduction less. However, when the SNF is reduced by 40%, the GS activity is lower than that of normal urea.

Effect of SNF on GOGAT activity in flag leaf of wheat. As shown in Figure 7C, the GOGAT activity in wheat flag leaves after anthesis decreased gradually with the advancement of the growth process. The GOGAT activity of the two-year experiment was at the same level as a whole. The differences between the treatments were basically the same, showing T1 > T2 > T3, CK > T4, and T1 was significantly higher than T2 most of the time. Still, in the end, there was no significant difference between the two. In 2021, the GOGAT activity of wheat flag leaves of CK, T1, T2, T3 and T4 decreased by 66.06, 59.51, 60.74, 62.26 and 65.66% respectively at 0–28 days after anthesis. In 2022, they decreased by 61.51,

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56.09, 55.50, 58.49 and 61.48%, respectively. It can be seen that the application of SNF can significantly increase the GOGAT activity of flag leaves after anthesis and reduce its decrease, but when the SNF is reduced by 40%, the GOGAT activity is lower than that of normal urea.

Effect of SNF on the expression of N transporter related genes in wheat roots

Effect of SNF on the expression of N transporter gene *TaNRT1.1* in wheat roots. Figure 8A shows the change of nitrate transporter gene *TaNRT1.1* expression in wheat roots after anthesis. The expression of *TaNRT1.1* under the treatment of SNF in each period was higher than that of the control, showing T1 > T2 > T3 > T4 > CK. Among them, there were no significant differences between T1 and T2 at 0 days and 21 days after anthesis in 2021. In 2022, there were significant differences among the treatments, and the maximum increase was at 14 days after anthesis. In 2021, T1, T2, T3 and T4 were increased by 3.95, 3.39, 2.71 and 1.91 times compared with CK on the 14 days after anthesis. In 2021–2022, it increased by 3.88, 3.32, 2.72 and 2.12 times, respectively. It can be seen that the application of SNF can significantly increase the expression of nitrate transporter gene *TaNRT1.1* in wheat roots.

Effect of SNF on the expression of ammonium transporter gene *TaAMT1.1* in wheat roots. Figure 8B shows the expression of the ammonium transporter gene *TaAMT1.1* in the roots of wheat after anthesis. Except for T4, the expression of *TaAMT1.1* under the SNF treatment at each stage was higher than that of the control, showing T1 > T2 > T3 > CK > T4. The difference between the treatments was significant, and the up-regulation was the largest at 14 days after anthesis. In 2021, T1, T2 and T3 were increased by 3.03, 2.53 and 1.80 times, respectively, compared with CK, and T4 was down-regulated by 0.19 times. From 2021 to 2022, they were up-regulated and down-regulated by 2.91, 2.42, 1.72, and 0.12 times, respectively.

Effect of SNF on N content of wheat

Effect of SNF on N content in wheat stem. As shown in Figure 9A, the total N content of wheat stems in each treatment gradually decreased with the increase of days after anthesis, and the difference between treatments gradually decreased. The total N content of wheat stems in 2021–2022 was at a high level. In 2021, the treatments showed T1 > T2 > T3 > CK > T4 at 0 days after anthesis, but there was no significant difference between T3 and CK. The difference between treatments in 2022 also gradually narrowed with the increase of days after anthesis. In 2021, the stem N content of CK, T1, T2, T3 and T4 wheat from anthesis to maturity decreased by 58.02, 64.24, 60.86, 59.51 and 59.10%, respectively. In 2022, they decreased by 56.83, 58.57, 61.12, 59.83 and 54.82%, respectively. The above results showed that the application of the same amount of SNF was beneficial to the accumulation of N content in wheat stems and the utilisation of N in wheat stems after anthesis, and the N content in stems did not decrease with the reduction of SNF.

Effect of SNF on N content in wheat leaf. As shown in Figure 9B, the total N content of wheat leaves in each treatment gradually decreased with the increase of days after anthesis. The total N content of wheat leaves in 2022 was at a high level, and the reduction rate was higher. The differences between the treatments in the two-year experiment were basically the same, and T1 and T2 at anthesis were significantly higher than that of other treatments. There were no significant differences among the treatments at 10 days after anthesis. At 20 days after anthesis, the differences among treatments were the largest. CK was significantly higher than T3 and T4 at maturity. In 2021, the leaf N content of CK, T1, T2, T3 and T4 wheat decreased by 59.87, 63.82, 63.76, 60.02 and 61.53% from anthesis to maturity, respectively. When the difference was the largest, T1, T2 and T3 were 22.97, 37.69 and 6.64% higher than CK, respectively, and T4 was 14.64% lower than CK; in 2022, it decreased by 64.78, 65.01, 65.44, 68.47 and 69.51%, respectively. When the difference was the largest, T1, T2 and T3 were 24.55, 6.77 and 38.06% higher than CK, respectively, and T4 was 15.92% lower than CK. The above results showed that the application of SNF was beneficial to the accumulation of N content in wheat leaves and the utilisation of N in wheat leaves after anthesis. However, when the amount of SNF was reduced by 40%, the leaf N content was lower than that of normal urea in some time periods.

Effects of SNF on N content of wheat spike. As shown in Figure 9C, the N content of wheat spike in each treatment gradually increased with the increase of days after anthesis. The differences between the treatments in the two-year experiment were basically the same. At the maturity of wheat, T1 was

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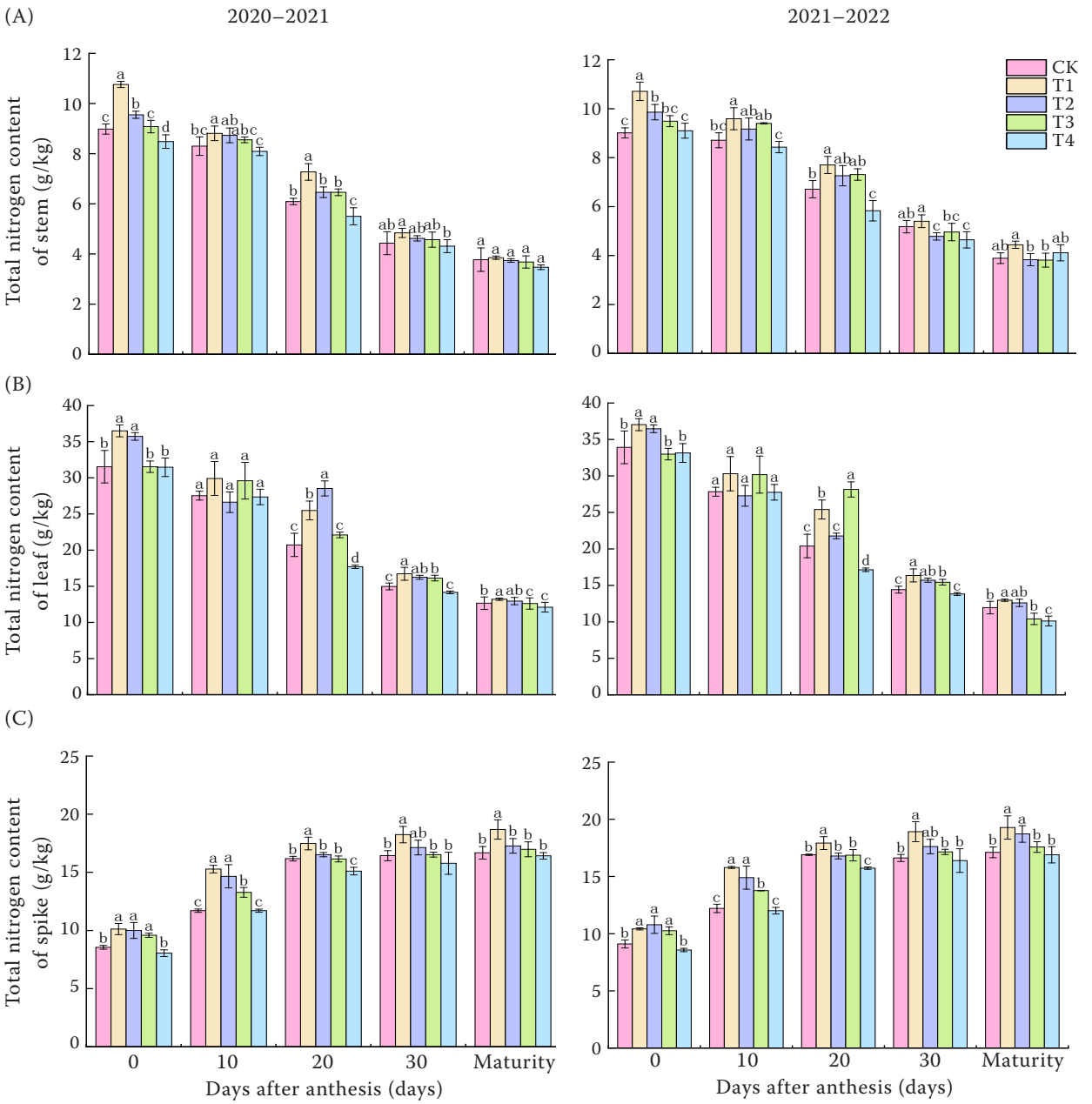

Figure 9. Total nitrogen (N) content of (A) wheat stem; (B) wheat leaf, and (C) wheat spike after anthesis. The type of mean comparison test was Duncan's new multiple-range method. The vertical bar represents the standard error, and the different letters above the error line represent the significant difference in the mean values of different treatments of the same measurement item ($P < 0.05$). CK – ordinary nitrogen fertiliser (299.86 kg N/ha); T1 – synergistic nitrogen fertilisers (SNF) (299.86 kg N/ha); T2 – SNF (239.89 kg N/ha); T3 – SNF (179.92 kg N/ha); T4 – SNF (119.94 kg N/ha)

significantly higher than other treatments in 2021, and T1 and T2 were significantly higher than other treatments in 2022. In 2021, the spike N content of CK, T1, T2, T3 and T4 wheat increased by 95.05, 84.65, 72.70, 77.09 and 104.02% from anthesis to maturity, respectively. Finally, the spike N content of T1, T2 and T3 wheat was 12.01, 3.52 and 1.76% higher than that of CK, respectively, and T4 was 1.60% lower than that of CK; in 2022, it increased by 87.92, 85.00, 73.57, 71.38 and 97.28%, respectively. Finally, the spike N content of T1, T2 and T3 was 12.66, 9.39 and 2.77% higher than CK's, respectively, and T4 was 1.23% lower than CK's. The above results showed that the application of SNF was beneficial

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Table 4. Nitrogen (N) accumulation and transport in wheat

	Treatment	Aboveground N content		N content in stems and leaves		N translocation amount in stems and leaves	N translocation rate in stems and leaves (%)
		at anthesis	at maturity	at anthesis	at maturity		
		(kg/ha)					
2020–2021	CK	114.972^{c}	151.625^{c}	105.574^{c}	26.432^{bc}	79.143^{c}	74.9^{b}
	T1	150.244^{a}	202.255^{a}	136.452^{a}	31.053^{a}	105.400^{a}	77.2^{a}
	T2	134.195^{b}	173.053^{b}	121.012^{b}	28.938^{ab}	92.074^{b}	76.1^{ab}
	T3	117.829^{c}	155.720^{bc}	105.608^{c}	25.115^{cd}	80.492^{c}	76.2^{ab}
	T4	106.634^{d}	140.286^{c}	97.992^{c}	22.549^{d}	75.443^{c}	77.0^{a}
2021–2022	CK	115.960^{c}	169.836^{cd}	101.182^{c}	26.564^{b}	74.618^{c}	73.7^{b}
	T1	158.075^{a}	215.021^{a}	141.785^{a}	33.175^{a}	108.610^{a}	76.6^{a}
	T2	139.891^{b}	195.375^{b}	122.304^{b}	31.958^{a}	90.346^{b}	73.9^{ab}
	T3	117.794^{c}	174.979^{c}	103.057^{c}	25.548^{b}	77.509^{c}	75.2^{ab}
	T4	107.750^{c}	160.890^{d}	96.13^{c}	24.45^{b}	71.680^{c}	74.6^{ab}

Different letters to the right of the numbers indicate significant differences in the means of different treatments for the same trait ($P < 0.05$). CK – ordinary nitrogen fertiliser (299.86 kg N/ha); T1 – synergistic nitrogen fertilisers (SNF) (299.86 kg N/ha); T2 – SNF (239.89 kg N/ha); T3 – SNF (179.92 kg N/ha); T4 – SNF (119.94 kg N/ha)

to the accumulation of N content in wheat spikes, with the most significant effects in the T1 and T2 treatments. In contrast, the N content of wheat spikes in the T4 treatment was lower than that of normal urea at some time after anthesis.

Effects of SNF on N accumulation and translocation of wheat in saline-alkali land

As shown in Table 4, the SNF significantly affects the N accumulation, N transport and N transport rate of wheat. In 2021, N accumulation at maturity showed T1 > T2 > T3 > CK > T4, but there was no significant difference between T2 and T3, and no significant difference between CK and T4. In 2022, N accumulation at maturity showed T1 > T2 > T3 > CK > T4, but the difference between CK and T4 was not significant. The amount of N transport in the two years was shown as T1 > T2 > T3 > CK > T4, but there was no significant difference between T3, CK, and T4. In 2021, T1, T2, and T3 were 33.18, 16.34, and 1.70% higher than CK, respectively, and T4 was 4.68% lower than CK. In 2022, T1, T2, and T3 were 45.55, 21.08, and 3.87% higher than CK, respectively, and T4 3.94% lower than CK. The N transport rate showed T1 > T4 > T3 > T2 > CK in 2021, but there was no significant difference between T1 and T4, and no significant difference between T2 and T3, and T1, T2, T3, and T4 were higher than CK by 3.07, 1.60, 1.74, and 2.80%, respectively. In 2022, it showed T1 > T3 > T4 > T2 > CK, but there was no significant difference between T2, T3 and T4, and T1, T2, T3 and T4 were higher than CK by 3.90, 0.27, 2.00 and 1.20%, respectively. The above results showed that the application of SNF was beneficial to the accumulation and transport of N in wheat. The N accumulation decreased with the decrease in the application amount of SNF, but the transport rate did not change significantly.

DISCUSSION

The presence of saline factors in saline land significantly affects the migration transformation, absorption, and utilisation of N in farmland. The ionic toxicity produced by salinisation causes osmotic stress in the crop root system, inhibits the absorption and utilisation of N by the crop, and reduces the utilisation rate of N fertiliser (Li et al. 2020). In this experiment, the N content of wheat and N accumulation in the aboveground part of the plant was significantly higher in the application of SNF treatments than CK, which may be due to the fact that synergists polymerised from active ingredients such as IA form a film on the surface of N fertiliser particles. IA can provide polymer chains with carboxylic side groups that are highly hydrophilic. In the presence of water-absorbing groups, the water-absorbent hydrogel molecules take up water and swell, the three-dimensional network structure of the polymer becomes enlarged, and the

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pores are filled with free water. After the N fertiliser particles are fully exposed to water in contact with water, the nutrient molecules are hydrolysed and dissolved in the three-dimensional network pores. When there is a concentration gradient difference between the inside and outside of the polymer, the nutrient molecules will be slowly released into the soil, which is the process of slow release of N fertilisers (Chen et al. 2023). Therefore, the application of SNF can slowly release soil nutrients, reduce N leaching, improve the nutrient status of saline and alkaline land, and is conducive to promoting plant N uptake. In this experiment, with the advancement of the fertility process after anthesis, the whole N content of the stem and leaf of wheat plants in all treatments gradually decreased, while the whole N content of spike gradually increased, the whole N content of spike reached the highest among all parts of wheat, and most of the N translocation amount and rate of wheat stems. Leaves were significantly higher than that of CK, which indicated that the appropriate amount of SNF could improve the crop seed yield by increasing the accumulation of nutrients and the transfer of nutrients to the seed grain after anthesis; this is similar to the findings of Liu et al. (2017). The N content of each part of the plant was not significantly different from that of the ONF most of the time under the reduced amount of SNF, indicating that the application of SNF is beneficial to the N uptake and utilisation of the post-flowering wheat plant, and promotes the N accumulation and transport of the post-flowering wheat, as well as reduces the amount of fertiliser applied and reduces the harm of fertiliser to the soil, which is also illustrated by the calculations of the amount of N accumulated in wheat and the amount of N transferred to the soil. However, N accumulation at anthesis and maturity of wheat in T4 treatment was slightly lower than with ONF in both years, which might be due to lower dry matter weight in case of 60% reduction in SNF.

The level of N in wheat plants is affected by a variety of factors. In order to investigate the N metabolism mechanism that causes the differences in N content, N accumulation and transport in wheat among treatments in this experiment, the expression of N uptake transporter protein genes in the root system and the activities of key enzymes for assimilation in the flag leaf of wheat were analysed to determine whether there were any differences. Nitrate transporter protein gene *TaNRT1.1* and ammonium transporter protein gene *TaAMT1.1* play important roles in N transport (Fang et al. 2021, Ijato et al. 2021). Nitrate (NO_3^-) is the main source of N in most plants, and nitrate and ammonium N are inorganic sources of N that are absorbed and utilised by plants (Dechorgnat et al. 2011), nitrate is mainly transported *via* the NO_3^- transport mechanism. Guo et al. (2014) found that nitrate transporter proteins play an important role in the response of wheat seedlings to N starvation under N starvation conditions, which led them to hypothesise that nitrate transporter protein genes can improve the efficiency of nitrate utilisation in plants. Ijato's experiments also mentioned that N starvation conditions triggered the expression of ammonium transporter protein genes (Ijato et al. 2021). In this experiment, by detecting the expression of nitrate transporter protein gene and ammonium transporter protein gene, it was found that the relative expression of the treatments between two years reached the highest level at 14 days after the anthesis of wheat. The treatment with the application of SNF was significantly higher than that of CK, which indicated that the SNF promoted the reproductive growth of wheat. The plant needed a large amount of N source up-regulated to support the seed grain formation, so the nitrate transporter protein gene and the ammonium transporter protein gene expression reached the highest level.

N uptake and utilisation by plants is a complex process, including N uptake, assimilation, transport and reuse of multiple links (Masclaux-Daubresse et al. 2010). The assimilation process determines how N is transferred to the seed and converted to yield and protein (Islam et al. 2021). NR is a rate-limiting enzyme in the plant N uptake and utilisation pathway that increases N metabolic activity and protein synthesis (Cheng et al. 2023). GS plays a central role in N metabolism and is essential for the assimilation of inorganic N. In contrast, GOGAT catalyses the conversion of glutamine to glutamate and provides glutamate for ammonium assimilation (Moore and Black 1979, Miflin and Habash 2002, Liu et al. 2021). Liu et al. (2021) found that the activity of GS/GOGAT in wheat leaves directly affects the efficiency of N assimilation, and up-regulation of GS/GOGAT leads to higher N utilisation. Ji et al. (2020) found that leaf photosynthetic N use efficiency during the maize kernel filling stage was significantly and positively correlated with NR, GS, and GOGAT, which was helpful to improve grain weight and yield under low N conditions. Related studies have shown that GS and GOGAT activities increase with increasing N levels but decrease with N starvation (Balotf et al. 2016, Shah

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et al. 2017). In this experiment, the activities of NR, GS and GOGAT in flag leaves of wheat treated with T1 and T2 were higher than those of CK at each stage, indicating that the application of SNF was beneficial to increase the N assimilation efficiency and use efficiency of wheat in saline-alkali soil. The activities of NR, GS and GOGAT were also significantly enhanced. However, the activity is lower than that of ONF in the case of SNF reduction, which will be continuously improved at a later stage to reduce the amount of fertiliser applied.

Photosynthesis is the basis for all plant life activities, and plant growth depends on it. Chlorophyll is an important photosynthetic pigment that absorbs and converts light energy, and its content is closely related to the photosynthetic capacity of plants (Weng et al. 2022). N promotes the increase of chlorophyll content and photosynthetic rate in plant leaves (Evans 2013). In this experiment, the chlorophyll content of wheat flag leaves after anthesis was maintained at a high level in T1 and T2, and the decrease in chlorophyll content was significantly reduced. This may be because N promotes chlorophyll synthesis by regulating the expression of genes involved in chlorophyll synthesis in leaves and suppresses the expression of genes involved in chlorophyll degradation, which plays an important role in maintaining photosynthesis (Chen et al. 2023). Zhang et al. (2017) also showed that an appropriate increase in N increased wheat's chlorophyll content and the wheat's photosynthetic performance. Photosynthesis can not be separated from the participation of water and CO_2. Saline and alkaline stress will affect the absorption of water by the root system of wheat. At the same time, the lack of water will also lead to a decrease in the water potential of the plant's chloroplasts, causing stomatal narrowing or even closure, which affects the absorption of CO_2 in the plant, leading to a weakening of photosynthesis (Sánchez-Romera et al. 2014). In this experiment, NPR, TR, and SC increased significantly at T1 and T2, and were more pronounced at a 20% reduction in SNF, which may be attributed to the slow release of N from the synergist, which provided sufficient N for late wheat development, thereby promoting dry matter accumulation, enhanced N availability, and maximum synthesis of chlorophyll content, leading to higher light capture and ultimately higher leaf NPR (Silvertooth et al. 2011). In addition, higher leaf NPR increased canopy development, which in turn led to higher SC. This is consistent with the findings of Perveen et al. (2021). Intercellular CO_2 concentration in the flag leaf of wheat could be maintained at a lower level for some time after flowering under the application of SNF, which might be due to the increased SC resulting in the elevated utilisation of CO_2 by the plant, which also resulted in the elevation of the NPR and the TR during the same period of time.

In this study, we investigated the effect of SNF on N metabolism of wheat after anthesis under saline conditions. The results showed that compared with ONF, the application of equal amounts of SNF significantly increased the activities of the key enzymes of N metabolism (NR, GS, and GOGAT) and up-regulated the expression of the root nitrate transporter protein gene, *TaNRT1.1*, and the ammonium transporter protein gene, *TaAMT1.1*, in the flag leaves of wheat. It increased the N accumulation of wheat plants and the N translocation of nutrient organs, promoted the dry matter accumulation of plants, facilitated the uptake and utilisation of N in wheat plants, and was able to effectively increase the NPR, TR, SC, and CO_2 utilisation rate of wheat flag leaves after anthesis. In conclusion, the results of this experiment showed that the application of SNF could promote the post-flowering growth and development as well as the N metabolism of saline wheat. It was also shown that it is possible to achieve fertiliser reduction in saline wheat production through the application of SNF, which improves FU and the net yield of wheat production.

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