Long-term effects of straw incorporation on seedling emergence and seedling quality of winter wheat on the Huang-Huai-Hai Plain

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Abstract: Rapid emergence and high-quality seedlings are an important basis for achieving high yields and high-quality grains in wheat production. It is not clear how long-term straw incorporation affects the emergence and quality of wheat seedlings in lime concretion black soil. Based on a 12-year long-term fixed-site experimental field, a three-year field experiment with wheat-maize rotation was conducted on the Huang-Huai-Hai Plain of China in 2020-2023 to assess the effect of straw incorporation on seedling emergence and seedling quality of wheat. The treatments comprised no-tillage wheat straw mulching and conventional tillage with maize straw return (T1), conventional tillage with maize straw return only (T2), and without straw incorporation (CK). The results showed that seedling emergence time (SET) and seedling emergence peak day (SEPD) in the T1 and T2 treatments were delayed compared with those of the CK. The SET was delayed (by 0.3-2.0 d), and the seedling emergence speed (SES) decreased (by 5.88%-25.01%), whereas the differences in emergence rate were not significant. The T1 and T2 treatments led to increases in leaf area, number of tillers per plant, and number of secondary roots at the six-leaf stage compared with those of the CK. Principal component analysis revealed that the first principal component that affected wheat seedling growth was dominated by the leaf area index and the net assimilation rate, whereas the leaf weight ratio mainly contributed to the second principal component. Straw incorporation delayed the wheat SET and the SEPD, and reduced the SES in the seedling emergence stage, but increased the leaf area, number of tillers per plant, and number of secondary roots in the seedling establishment stage, thereby promoting the development of strong seedlings. This study provides theoretical support for the efficient utilization of straw resources and the improvement of the quality of wheat seedlings on the Huang-Huai-Hai Plain.

Keywords: straw return, *Triticum aestivum* L., seedling quality, principal component analysis, lime concretion black soil
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1 Introduction

Wheat (*Triticum aestivum* L.), a member of the Gramineae family, is among the most important crops worldwide[1]. In 2022, the global wheat production area was 2.19×10^8 hm^2 with a yield of 8.08×10^8 t. China's wheat production (1.18×10^8 t) was ranked first in the world, accounting for approximately 17% of the global wheat yield[2]. The Huang-Huai-Hai Plain is the most important wheat-producing area in China, contributing approximately 70% of the wheat yield of China, and the wheat-maize (*Zea mays* L.) rotation system is the dominant cropping system practiced on the plain[3]. In recent decades, with the increasing grain production, the amount of crop straw produced has synchronously increased[4]. Crop straw, as a low-cost and readily obtainable energy source, contains abundant nitrogen (N), phosphorus (P), potassium (K), and trace elements. Crop straw is used for various purposes, such as straw incorporation, straw storage, straw-based raw material conversion, and biofuel production. Straw incorporation, applied as a protective tillage measure and as an efficient and green resource-recovery method, plays a crucial role in maintaining the soil moisture content, improving crop water-use efficiency, and preserving the ecological environment of farmland, and has been widely promoted and adopted throughout the world[5]. Extensive research has been conducted on improving soil health and increasing crop yields through straw return, with particular focus on changes in soil physical and chemical properties after straw return[6], changes in soil microbial communities, and crop growth and yield[7].

Lime concretion black soil, as an ancient cultivated soil and a relatively moderate- to low-yielding soil type in China, has a highly localized distribution in China's important grain, fruit, and vegetable produce areas[8]. The area of lime concretion black soil in China is approximately 4.0×10^6 hm^2, accounting for approximately 3.4% of the total planting area of grain crops. Given its low fertility, sticky and heavy texture, as well as susceptibility to waterlogging and drought, lime concretion black soil severely restricts the production and development of crops[8]. Straw incorporation is a

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promising method to improve the soil fertility and promote increased grain yield at the local scale. Previous studies have indicated that fertilization in combination with crop residue application stimulates the release of labile organic carbon and may be a useful approach for sustainable production in lime concretion black soils[9]. Straw incorporation with reduced fertilizer application is beneficial for increasing the content, proportion, and carbon pool management index of active organic carbon in lime concretion black soil[10]. Applying deep tillage and straw return to the field in the lime concretion black soil area promotes the accumulation of organic carbon and the formation of macropores in the >10-40 cm soil layer. The application of biochar under conventional fertilization promotes the accumulation of total organic carbon, and the availability of P and K in the soil[11].

Although straw incorporation has a positive impact on soil structure and fertility, it poses a threat to the rapid emergence of wheat seedlings and the development of high-quality seedlings owing to factors such as an increase in soil porosity and physical obstruction[12]. Especially in recent years, against the backdrop of global climate change, extreme meteorological events (such as late-spring coldness and dry-hot winds) have increased in frequency, posing a challenge to sustaining food security in China[13]. A vigorous, healthy seedling is not only resistant to damage from extreme weather before winter, but also has an improved capability to resist abiotic stresses, such as late-spring coldness or dry-hot winds, at subsequent developmental stages[14]. Therefore, to address the problems relating to seedling emergence caused by straw incorporation in lime concretion black soil, such as weak yellowed seedlings and seedling mortality, and obstruction of seedling emergence and growth, a long-term straw-return experiment is in progress in the lime concretion black soil area. The present study investigated the characteristics of winter wheat seedling emergence and quality under three straw management regimes. The objective is to provide a technical and theoretical foundation to ensure the development of strong seedlings over winter and contribute to national food security under global climate change.

2 Materials and methods

2.1 Study site description

The present field experiment was conducted as part of a 12-year long-term experiment, which was initiated in 2008 at the Scientific Observation and Experimental Station of Crop Cultivation in East China, Ministry of Agriculture, China, in Mengcheng County, Bozhou City, Anhui Province (33°09 ′N, 116°32′E, 28 m.a.s.l.). The study was performed in the winter wheat growing seasons of 2020-2021, 2021-2022, and 2022-2023. The study area has a warm-temperate semi-humid monsoon climate, with an average annual temperature of 14.8°C and average annual precipitation of 732.63 mm, mainly concentrated in June-August. The frost-free period is 214 days and the average annual sunshine duration is 2410 h. The soil is a typical lime concretion black soil with the following chemical properties in the 0-20 cm layer at the beginning of the experiment (in 2008): 12.46 g/kg organic matter, 0.99 g/kg total N, 80.20 mg/kg alkali hydrolyzable N, 15.40 mg/kg available P, and 100.30 mg/kg available K. The monthly average rainfall and temperature in the wheat growing seasons of 2020-2021, 2021-2022, and 2022-2023 are shown in Figure 1. The total rainfall was 448.00 mm, 253.3 mm, and 493.8 mm in the 2020-2021, 2021-2022, and 2022-2023 wheat growing seasons, respectively.

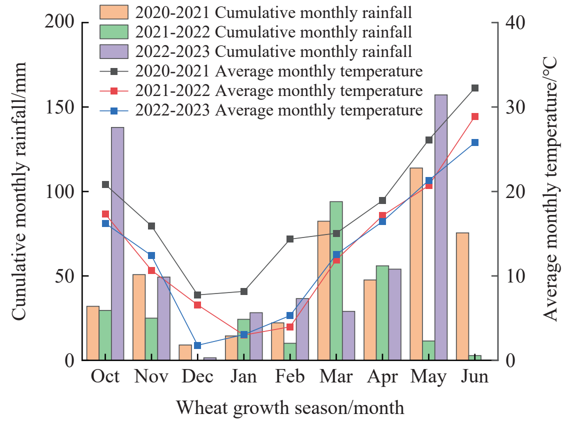


Figure 1 Cumulative monthly rainfall and average monthly temperature for 2020-2021, 2021-2022, and 2022-2023 wheat growing seasons at experiment site

2.2 Experimental design and field management

This is a long-term in situ positioning experiment that started in 2008, and the data were collected from 2020 to 2023, which represent years 12, 13, and 14 of the long-term trial. The experiment included three treatments: 1) without straw incorporation (CK), 2) wheat and maize straw were returned to the field (T1), and 3) only maize straw was returned to the field (T2). Each treatment was applied with three replicates in field plots of area 43.2 m^2 (8.0 m×5.4 m). The wheat and maize straw were crushed into pieces less than 10 cm in length. Wheat straw was spread evenly over the soil surface as a mulch without tillage, whereas maize straw was returned to the 0-20 cm soil layer using a large rotary tiller (Lutong-1304, Luoyang, China). If no straw was returned to the field, the whole residual plants were artificially removed from the experimental plots. The experiment site and overview of the straw return to the field for each treatment is shown in Figure 2.

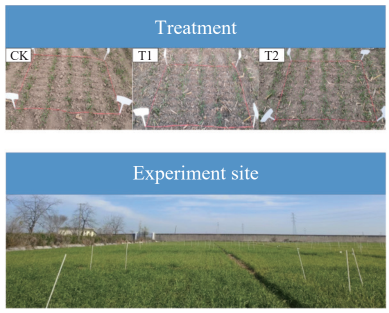


Figure 2 Experiment site (33°09′N, 116°32′E) and overview of straw return to field for each treatment

The experimental site was situated in a wheat-maize rotation area. The wheat variety tested was Yan Nong 19, and the maize variety was Zheng Dan 958. Wheat was sown on October 15, 2020, October 19, 2021, and October 19, 2022, and harvested on June 1, 2021, June 2, 2022, and June 3, 2023, respectively. The seeding rate of wheat was 187.5 kg/hm^2. In the experiment, agricultural potassium sulfate compound fertilizer (N:P_2O_5:K_2O=15:15:15, total nutrients>45%) and urea (N≥46%) were applied. The doses of N, P, and K in the chemical fertilizers were 240 kg N/hm^2, 90 kg P_2O_5/hm^2, and 90 kg K_2O/hm^2. Of the total amount, 55% of N was applied as a basal fertilizer and 45% was applied as a topdressing, whereas all P and K were applied before sowing. Field management followed local conventional cultivation practices. Other management practices were identical to those of the high-yield field.

2.3 Sampling and measurement

2.3.1 Indices related to seedling emergence

On the sixth day after the wheat seeds were sown, 1 m² sample plots were selected for each treatment (repeated three times), and the emergence of seedlings was recorded at 14:00 daily (in 2020, 2021, and 2022). When the number of wheat seedlings in a 1 m² plot was less than 10 on the first and second days, emergence was considered complete. The seedling emergence time (SET) refers to the duration from the beginning of seedling emergence to stable emergence. The seedling emergence peak day (SEPD) refers to the day on which the greatest number of seedlings emerged in a single day after the start of emergence, and the seedling emergence speed (SES) is the reciprocal of the emergence time[15].

2.3.2 Emergence rate

At 20 d after sowing, the number of seedlings in the three 1 m² plots of each treatment were recorded, and the emergence rate was calculated based on the number of seeds sown and the 1000-grain weight as follows:

$$\text{Emergence rate} = \frac{\text{Number of seedlings}}{\text{Number of sown seeds}} \times 100\% \tag{1}$$

2.3.3 Seedling characteristics

At the three- and six-leaf stages of the wheat growing season from 2021 to 2023, 15 plants were sampled from each plot to determine the number of tillers, age of leaves, number of secondary roots, and sheath thickness. The height, length, and maximum width of the wheat seedlings were measured using a ruler and the leaf area index (LAI) was calculated[16]. Subsequently, the plants were divided into leaves, leaf sheaths, and roots, and placed in a constant-temperature air-drying oven (DHG-240L, Anhui, China) at 105°C for 30 min to kill the green tissues. After drying at 65°C to a constant weight, the dry weight of each organ was measured.

2.3.4 Growth analysis

Based on the measured leaf area and the dry weights, plant growth parameters were calculated using the following formulas:

$$\text{LAR} = \frac{(\ln W_2 - \ln W_1) \times (L_2 - L_1)}{(W_2 - W_1) \times (\ln L_2 - \ln L_1)} \tag{2}$$

$$\text{SLA} = \frac{L}{L_w} \tag{3}$$

$$\text{LWR} = \frac{L_w}{w} \tag{4}$$

$$\text{RGR} = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \tag{5}$$

$$\text{NAR} = \frac{(\ln L_2 - \ln L_1) \times (W_2 - W_1)}{(L_2 - L_1) \times (t_2 - t_1)} \tag{6}$$

where, LAR is leaf area ratio, cm²/g; SLA is specific leaf area, cm²/g; LWR is leaf weight ratio; RGR is relative growth rate, g/(g·d); NAR is net assimilation rate, g/(m²·d); W, L, and L_W are plant dry weight, leaf area, and leaf dry weight, respectively; t_1 is the time to reach the three-leaf stage; t_2 is the time to reach the six-leaf stage; and W_1, W_2, L_1, and L_2 are the dry weight and leaf area of wheat plants during the three-leaf stage (t_1) and six-leaf stage (t_2) of seedlings, respectively.

2.3.5 Grain yield

At the maturity stage, the grain yields of wheat plants harvested from each plot were determined. The 1 m² sample plots were selected for each treatment and the grain yield of wheat was measured (repeated three times). The grain yield (GY; kg/hm²) was measured for natural air-dried grains.

2.3.6 Correlation analysis and principal component analysis

Pearson's correlation analysis was performed to analyze the correlations between the growth indicators at the six-leaf stage and grain yield. Principal component analysis was performed to identify the main factors affecting seedling quality. The relevant indicators used to evaluate the seedling quality under each straw-return treatment were converted as follows:

$$\text{Seedling quality evaluation coefficient} = \frac{\text{Treatment value}}{\text{Control value}} \tag{7}$$

A principal component analysis was performed on the calculated seedling quality evaluation coefficients.

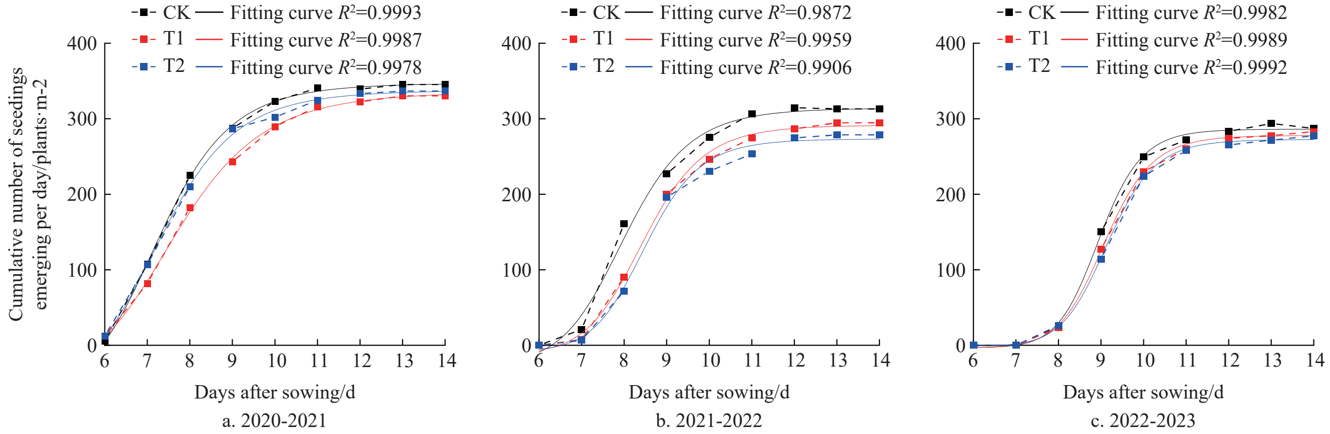
2.4 Data analysis

Data are expressed as the mean±standard error (n=3). All statistical analyses were performed with IBM SPSS Statistics 26.0 (IBM Corporation, Armonk, NY, USA). All charts were plotted using Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA) and OriginPro 2021 (Origin Lab Corporation, Northampton, MA, USA). The correlation heatmap was plotted using R-4.4.1. Duncan's new multiple range test was used for assessment of significance among the means (p<0.05).

3 Results and discussion

3.1 Cumulative number of seedlings emerging per day

As shown in Figure 3, in the three study years, with progression from the sowing time, the cumulative number of wheat seedlings per unit area conformed to a sigmoid curve under each straw-return treatment. The coefficients of determination (R^2) for the equations



Note: R^2 represents the fitting coefficient of the equation. The closer the value of R^2 is to 1, the higher the degree of equation fitting.

Figure 3 Effect of straw return on cumulative number of wheat seedlings emerging per day in each growing season representing the relationship between the number of seedlings on a single day with the number of days after sowing for each treatment were all greater than 0.99, indicating that the logistic curve equation adequately represented the variation in cumulative numbers of seedlings on a single day with the number of days post-sowing.

3.2 Seedling emergence time and emergence peak day

As listed in Table 1, straw return distinctly affected the SET and SEPD. In 2020, the SET was delayed by 0.3-2.0 d compared with that of the CK. Among the treatments, SET in T2 was significantly delayed by 2 d compared with the CK. No significant difference in the SEPD was observed, which was reached on the seventh day after sowing. In 2021, the time of wheat SET and SEPD were delayed by straw return. Compared with the CK, the wheat SET was delayed by 0.7-1.0 d and the SEPD was delayed by 1.0 d. In 2022, compared with the CK, the wheat SET was delayed by 0.3-0.7 d and the SEPD was delayed by 0.4 d in T2. These results showed that the straw-return treatments prolonged the SET and delayed SEPD of wheat seedlings.

Interaction analysis showed that the interaction effect between years and treatments was not significant. The year was not significantly correlated with SET or SEPD, but the different treatments had a significant impact. These results indicated that the SET and SEPD of wheat were mainly affected by the straw-return treatment.

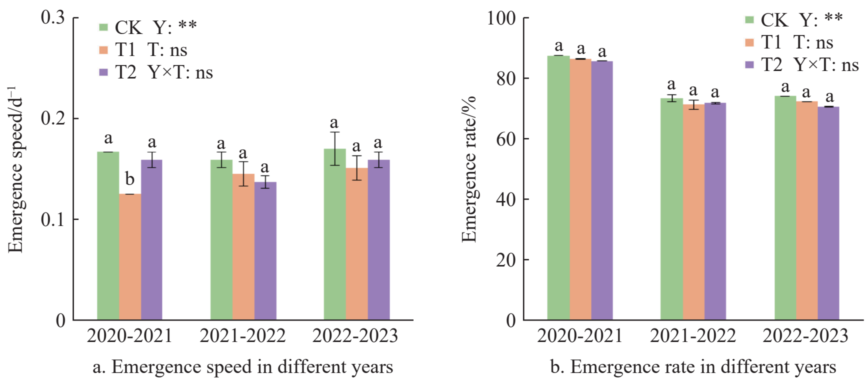
3.3 Emergence speed and emergence rate

Straw incorporation affected the seedling emergence speed and emergence rate, as seen in Figure 4. The emergence speeds of T1 and T2 were less than that of the CK in all three years. In 2020, the emergence speed of T1 was significantly reduced by 25.01% compared with the CK. In 2021, compared with the CK, the emergence speeds of T1 and T2 were lower than CK, but there was no significant difference among the treatments. In 2022, no significant differences in emergence speed were observed among the treatments. Straw incorporation had a relatively small impact on the emergence rate of wheat, as shown in Figure 4b. No significant difference in the emergence rate among the treatments was detected in any of the three years.

Table 1 Effects of straw return on seedling emergence time and emergence peak day of wheat

Year	Treatment	SET/d	SEPD/d
2020-2021	CK	6.0±0.0^b	7.0±0.0^a
	T1	8.0±0.0^a	7.0±0.0^a
	T2	6.3±0.3^b	7.0±0.3^a
2021-2022	CK	6.3±0.3^a	8.0±0.0^a
	T1	7.0±0.6^a	9.0±0.0^a
	T2	7.3±0.3^a	9.0±0.0^a
2022-2023	CK	6.0±0.6^a	8.3±0.3^a
	T1	6.7±0.3^a	8.3±0.3^a
	T2	6.3±0.3^a	8.7±0.3^a
	Y	ns	ns
	T	**	*
	Y×T	ns	ns

Note: Seedling emergence time (SET) refers to the period from the beginning of wheat emergence to stable emergence. The seedling emergence peak day (SEPD) refers to the day when the number of seedlings emerging in a single day was the highest after emergence began. Within a column, means with different lowercase letters are significantly different (p<0.05), and means that share a lowercase letter are not significantly different (p>0.05). Analysis of interaction results were shown (*, **=significant at 0.05, 0.01 probability levels; ns=not significant). The "Y" refers to year; the "T" refers to treatment. Same as below.



Note: In the figures, means with different lowercase letters are significantly different (p<0.05), and means that share a lowercase letter are not significantly different (p>0.05). Analysis of interaction results are shown (*, **=significant at 0.05, 0.01 probability levels; ns=not significant). The "Y" refers to year; the "T" refers to treatment. Same as below.

Figure 4 Effects of straw return on emergence speed and emergence rate of winter wheat seedlings

3.4 Characteristics of wheat seedlings at three-leaf stage

Straw incorporation increased the height, sheath thickness, and leaf area of winter wheat seedlings at the three-leaf stage, as seen in Table 2. Compared with the CK, the seedling heights of T1 and T2 increased by 8.52% and 11.17% in 2021, and 6.92% and 1.63% in 2022, respectively. The sheath thicknesses increased by 9.88% and 13.44% in 2021, and 29.57% and 21.74% in 2022, respectively. The leaf areas per plant increased by 11.23% and 11.15% in 2021, and 29.76% and 3.99% in 2022, respectively. However, compared with the CK, T1 and T2 significantly reduced leaf age by 5.09% and 5.09% in 2021, respectively. These results showed that straw incorporation promoted wheat growth and development at the three-leaf stage, and the effects on seedling height, sheath thickness, and leaf area were significant (p<0.05).

The dry-matter weight is a direct reflection of the accumulation of photosynthetic substances. Straw incorporation had an obvious impact on the accumulation of dry matter in wheat, as recorded in Table 2. From 2021 to 2022, no significant differences in the dry-matter weight of wheat leaves were observed among the treatments, but the dry weight of the leaf sheaths in T1 and T2 increased significantly, by 44.0% and 48.0%, respectively, compared with the CK. From 2022 to 2023, T1 significantly increased the dry weight of leaves by 15.0% compared with the CK, whereas the difference in sheath dry weight was not significant. T2 significantly increased the root dry weight and root-canopy ratio by 50.0% and 50.0%, respectively, compared with those of the CK. These results indicated that straw incorporation mainly promoted the accumulation of dry matter in wheat leaves to enhance the dry matter in wheat. Among the treatments, T1 had the most strongly significant effect on promoting the dry-matter weight of wheat leaves.

Table 2 Effects of straw return on morphology of wheat seedlings at three-leaf stage

Year	Treatment	Seedling height/cm	Leaf age	Leaf sheath thickness/mm
2021-2022	CK	24.54±0.52^{b}	3.34±0.03^{a}	2.53±0.05^{b}
	T1	26.63±0.41^{a}	3.17±0.01^{b}	2.78±0.01^{a}
	T2	27.28±0.26^{a}	3.17±0.04^{b}	2.87±0.01^{a}
2022-2023	CK	22.70±0.25^{a}	3.52±0.06^{a}	2.30±0.15^{a}
	T1	24.27±0.29^{a}	3.69±0.01^{a}	2.98±0.12^{a}
	T2	23.07±0.72^{a}	3.54±0.07^{a}	2.80±0.27^{a}
	Y	**	**	ns
	T	**	ns	**
	Y×T	*	**	ns

Year	Treatment	Leaf area per plant/cm^{2}	Number of secondary roots	Dry matter of leaf/g
2021-2022	CK	11.75±0.08^{a}	1.39±0.24^{b}	0.055±0.002^{a}
	T1	13.07±0.11^{a}	2.28±0.24^{a}	0.059±0.002^{a}
	T2	13.06±1.17^{a}	1.33±0.19^{b}	0.060±0.003^{a}
2022-2023	CK	11.29±0.47^{b}	1.67±0.88^{a}	0.059±0.002^{ab}
	T1	14.65±0.46^{a}	2.67±0.33^{a}	0.069±0.001^{a}
	T2	11.74±0.56^{b}	2.33±0.33^{a}	0.056±0.005^{b}
	Y	ns	ns	ns
	T	**	ns	ns
	Y×T	ns	ns	ns

Year	Treatment	Dry matter of leaf sheath/g	Dry matter of root/g	Root-canopy ratio
2021-2022	CK	0.025±0.001^{b}	0.020±0.000^{a}	0.29±0.01^{a}
	T1	0.036±0.002^{a}	0.022±0.005^{a}	0.24±0.06^{a}
	T2	0.037±0.003^{a}	0.017±0.003^{a}	0.18±0.02^{a}
2022-2023	CK	0.023±0.001^{a}	0.012±0.001^{b}	0.14±0.01^{b}
	T1	0.029±0.000^{a}	0.012±0.001^{b}	0.11±0.01^{b}
	T2	0.024±0.003^{a}	0.018±0.000^{a}	0.21±0.02^{a}
	Y	**	*	**
	T	**	ns	ns
	Y×T	ns	ns	*

3.5 Characteristics of wheat seedlings at six-leaf stage

Straw incorporation increased the seedling height, leaf age, tiller number per plant, and leaf area per plant of winter wheat at the six-leaf stage, as noted in Table 3. From 2021 to 2023, T1 and T2 showed a higher main stem height, largest tiller height, second largest tiller height, third largest tiller height, and fourth largest tiller height compared with those of the CK. The trend among treatments for main stem leaf age, largest tiller leaf age, second largest tiller leaf age, third largest tiller leaf age, and fourth largest tiller leaf age was T1>T2>CK.

Compared with the CK, T1 and T2 significantly increased the sheath thickness by 20.71% and 11.81% in 2021, respectively, and the number of tillers per plant was significantly increased by 38.57% and 7.85% in 2021, respectively, as is evident in Table 4. In each year, the trend for the main stem leaf area of wheat seedlings at the six-leaf stage was T1>CK>T2. The largest tiller leaf area, second largest tiller leaf area, and third largest tiller leaf area showed the trend T1>T2>CK. T1 and T2 increased the area of the first and third largest tiller leaves during 2021–2023 compared with those of the CK. After wheat seedlings entered the six-leaf stage, straw incorporation on the morphological indicators of the seedlings had a promotive effect, indicating that the negative impact of straw incorporation on the growth and development of wheat seedlings at the three-leaf stage could be transformed into a positive effect at the six-leaf stage, promoting the development of strong wheat seedlings that safely passed through the overwintering stage, thus laying the foundation for stable and high grain yields.

Table 3 Effects of straw incorporation on morphology of wheat seedlings at six-leaf stage

Year	Treatment	Main stem seedling height/cm	Largest tiller seedling height/cm	Second largest tiller seedling height/cm
2021-2022	CK	25.67±0.17^{b}	18.33±0.09^{b}	18.37±0.30^{b}
	T1	28.90±0.42^{a}	21.17±0.09^{a}	21.10±0.31^{a}
	T2	26.07±0.32^{b}	20.57±0.28^{a}	20.50±0.49^{a}
2022-2023	CK	30.43±0.60^{c}	26.27±0.43^{b}	25.37±0.58^{b}
	T1	35.13±0.03^{a}	28.80±0.49^{a}	28.73±0.30^{a}
	T2	32.13±0.60^{b}	26.43±0.68^{b}	26.97±0.82^{ab}
	Y	**	**	**
	T	**	**	**
	Y×T	ns	ns	ns

Year	Treatment	Third largest tiller seedling height/cm	Fourth largest tiller seedling height/cm	Main stem leaf age
2021-2022	CK	15.50±0.21^{b}	8.07±0.38^{a}	5.84±0.24^{a}
	T1	18.43±0.54^{a}	8.83±0.33^{a}	6.22±0.03^{a}
	T2	17.70±0.56^{a}	8.83±0.03^{a}	6.15±0.08^{a}
2022-2023	CK	19.53±0.53^{b}	-	6.15±0.10^{b}
	T1	21.63±0.48^{a}	-	6.43±0.05^{a}
	T2	21.40±0.55^{a}	-	6.16±0.05^{b}
	Y	**	**	ns
	T	**	ns	*
	Y×T	ns	ns	ns

Year	Treatment	Largest tiller leaf age	Second largest tiller leaf age	Third largest tiller leaf age
2021-2022	CK	3.36±0.08^{a}	2.58±0.04^{b}	1.64±0.03^{a}
	T1	3.52±0.03^{a}	2.74±0.02^{a}	1.68±0.01^{a}
	T2	3.42±0.00^{a}	2.59±0.01^{b}	1.67±0.02^{a}
2022-2023	CK	3.30±0.03^{b}	2.48±0.12^{a}	1.63±0.09^{a}
	T1	3.78±0.05^{a}	2.28±0.28^{a}	1.85±0.07^{a}
	T2	3.37±0.18^{b}	2.60±0.11^{a}	1.66±0.12^{a}
	Y	ns	ns	ns
	T	**	ns	ns
	Y×T	ns	ns	ns

Year	Treatment	Fourth largest tiller leaf age	Number of tillers per plant	Number of primary tillers
2021-2022	CK	0.17±0.02^{a}	4.33±0.33^{b}	3.67±0.33^{a}
	T1	0.22±0.02^{a}	6.00±0.58^{a}	3.67±0.33^{a}
	T2	0.20±0.05^{a}	4.67±0.33^{ab}	3.67±0.33^{a}
2022-2023	CK	-	3.33±0.33^{a}	-
	T1	-	3.33±0.33^{a}	-
	T2	-	3.33±0.33^{a}	-
	Y	**	**	ns
	T	ns	ns	ns
	Y×T	ns	ns	ns

Note: "-" indicates that no data are available for the item.

The dry weight of the main stem leaf, the largest tiller leaf, the second largest tiller leaf, and the third largest tiller leaf were higher than those of the CK, as shown in Table 5. The dry weight of the main stem leaf, the largest tiller leaf sheaths, the second largest tiller leaf sheaths, and the third largest tiller leaf sheaths showed the same trend as the dry weight of the leaves, and all were higher than those of the CK. In 2021, the dry weight of the root of T2 was significantly higher than that of the CK, but no significant effect was detected in 2022. The root-canopy ratio was highest in T2 and lowest in T1 in 2022. These results showed that straw incorporation could increase the dry weight of wheat plants at the six-leaf stage, which was conducive to the development of strong seedlings before winter. The overall trend in dry-matter weight among the straw-return treatments was T1>T2>CK. The annual straw incorporation of the wheat-maize rotation had the most significant promotive effect on the dry weight of wheat seedlings at the six-leaf stage.

Table 4 Effects of straw return on morphology of wheat seedlings at six-leaf stage (continued)

Year	Treatment	Leaf sheath thickness/mm	Number of secondary roots	Main stem leaf area/cm²
2021-2022	CK	6.18±0.13[c]	9.00±0.58[a]	21.31±0.86[b]
	T1	7.46±0.16[a]	10.00±0.58[a]	27.61±0.74[a]
	T2	6.91±0.16[b]	9.33±0.33[a]	20.28±1.28[b]
2022-2023	CK	6.27±0.24[a]	7.67±0.88[a]	29.44±0.40[a]
	T1	6.67±0.28[a]	8.67±0.67[a]	30.41±2.59[a]
	T2	7.23±0.34[a]	7.67±0.88[a]	28.86±1.22[a]
	Y	ns	ns	*
	T	**	**	ns
	Y×T	ns	ns	ns
		Largest tiller leaf area/cm²	Second largest tiller leaf area/cm²	Third largest tiller leaf area/cm²
2021-2022	CK	6.68±0.15[b]	6.69±0.09[b]	3.15±0.17[a]
	T1	13.50±0.60[a]	9.54±0.23[a]	3.58±0.00[a]
	T2	7.69±0.53[b]	6.92±0.38[b]	3.24±0.16[a]
2022-2023	CK	12.03±1.07[a]	9.02±0.57[a]	2.81±0.09[a]
	T1	15.32±0.52[a]	9.76±0.74[a]	3.20±0.32[a]
	T2	12.83±1.28[a]	8.65±0.52[a]	3.22±0.39[a]
	Y	**	**	ns
	T	*	**	ns
	Y×T	ns	ns	ns
		Dry weight of main stem leaves/g	Dry weight of the largest tiller leaf/g	
2021-2022	CK	0.16±0.010[a]	0.04±0.010[b]	-
	T1	0.19±0.010[a]	0.07±0.000[a]	-
	T2	0.17±0.000[a]	0.04±0.010[b]	-
2022-2023	CK	0.15±0.004[ab]	0.06±0.002[a]	-
	T1	0.20±0.002[a]	0.07±0.005[a]	-
	T2	0.07±0.05[b]	0.06±0.007[a]	-
	Y	ns	**	-
	T	*	**	-
	Y×T	ns	ns	-
		Dry weight of the second largest tiller leaf/g	Dry weight of the third largest tiller leaf/g	-
2021-2022	CK	0.06±0.001[a]	0.02±0.001[b]	-
	T1	0.06±0.003[a]	0.03±0.001[a]	-
	T2	0.06±0.001[a]	0.02±0.000[b]	-
2022-2023	CK	0.05±0.002[b]	0.01±0.006[b]	-
	T1	0.07±0.002[a]	0.03±0.001[a]	-
	T2	0.05±0.005[b]	0.02±0.002[ab]	-
	Y	ns	*	-
	T	**	**	-
	Y×T	**	ns	-

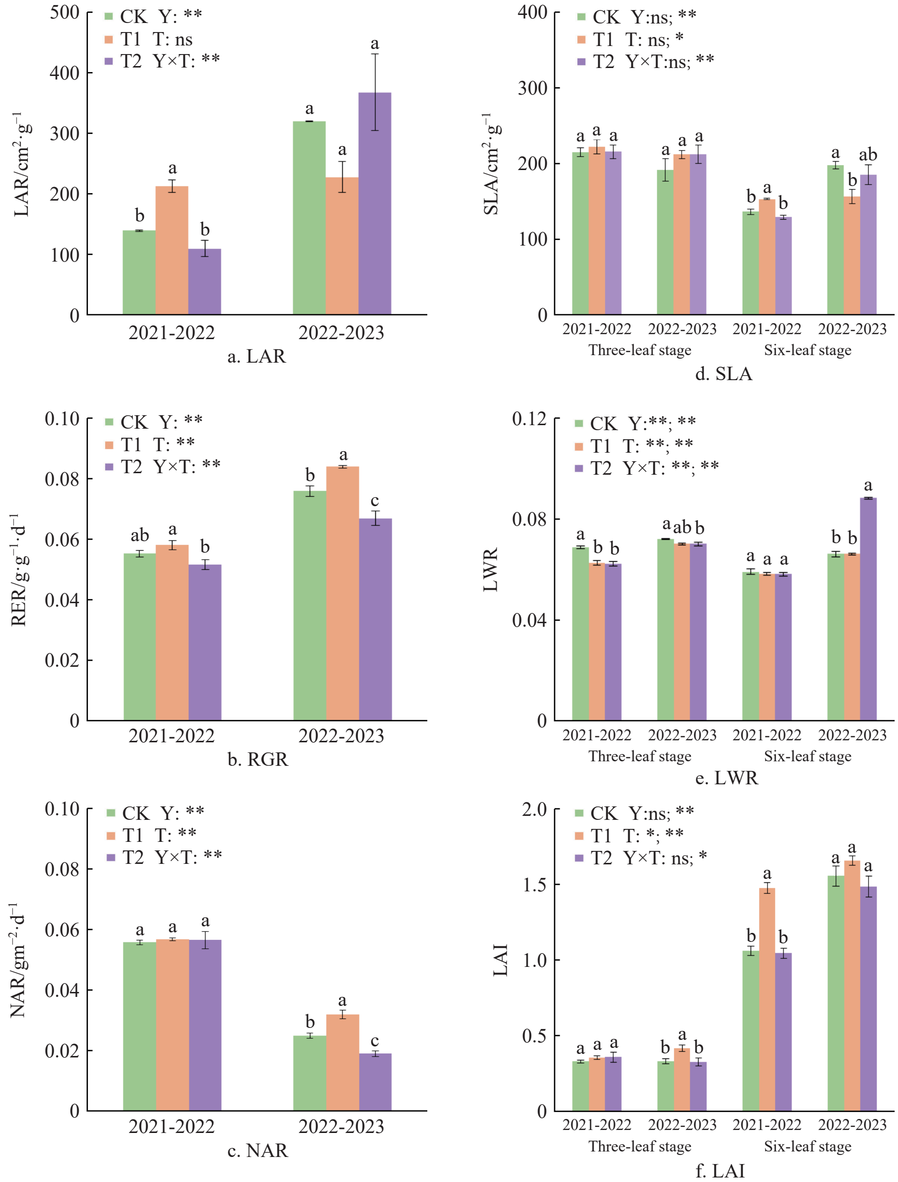
Table 5 Effects of straw incorporation on morphology of wheat seedlings at six-leaf stage (continued)

Year	Treatment	Dry weight of main stem leaf sheath/g	Dry weight of the largest tiller leaf sheath/g	Dry weight of the second largest tiller leaf sheath/g
2021-2022	CK	0.09±0.002[c]	0.03±0.002[b]	0.05±0.003[a]
	T1	0.12±0.002[a]	0.05±0.001[a]	0.05±0.010[a]
	T2	0.10±0.002[b]	0.04±0.004[b]	0.05±0.003[a]
2022-2023	CK	0.07±0.006[b]	0.03±0.002[a]	0.03±0.001[b]
	T1	0.09±0.004[a]	0.04±0.002[a]	0.04±0.001[a]
	T2	0.07±0.004[b]	0.03±0.004[a]	0.03±0.004[b]
	Y	**	*	**
	T	**	**	*
	Y×T	*	ns	ns
		Dry weight of the third largest tiller leaf sheath/g	Root/g	Root-canopy ratio
2021-2022	CK	0.03±0.002[a]	0.07±0.00[b]	0.14±0.00[b]
	T1	0.03±0.001[a]	0.08±0.00[ab]	0.13±0.00[b]
	T2	0.03±0.001[a]	0.09±0.01[a]	0.17±0.02[a]
2022-2023	CK	0.001±0.002[b]	0.04±0.00[a]	0.09±0.01[ab]
	T1	0.021±0.002[a]	0.04±0.01[a]	0.06±0.01[b]
	T2	0.02±0.001[ab]	0.04±0.00[a]	0.12±0.01[a]
	Y	**	**	**
	T	**	*	**
	Y×T	ns	ns	ns

3.6 Seedling growth analysis

The analysis of the growth indicators of wheat seedlings from the three- to six-leaf stage significantly affected by straw return is shown in Figure 5. The trend of LAR performance in different straw returning was opposite over the past two years, and the interaction effect reached a very significant level, as seen in Figure 5a. The RGR of wheat seedlings from the three- to six-leaf stage is shown in Figure 5b. The effects of year and treatment on RGR reached a very significant level, respectively, and the interaction effect also reached a very significant level. The year had significant effects on the NAR in 2021-2022, while the different treatments had no significant effects in Figure 5c. The impact of year and treatment on NAR reached a highly significant level in 2022-2023. The interaction effect also reached a highly significant level.

From 2021 to 2023, the trend in SLA performance during the three-leaf stage of wheat was basically the same, with T2>T1>CK, and the interaction effect did not reach a significant level. The SLA of the six-leaf stage showed different trends in the two growth seasons, but the impact of year on it reached a highly significant level, and the interaction effect also reached a highly significant level in Figure 5d. The LWR performance trend in the three-leaf stage of wheat seedlings is basically consistent in the two wheat seasons of 2021-2023 in Figure 5e, both with CK>T1>T2. The impact of year and treatment on LWR in the three-leaf stage was extremely significant, and the interaction effect was also extremely significant. The impact of LWR in the six-leaf stage was significant from 2022-2023, and the impact of year and treatment on LWR in the six-leaf stage was extremely significant. The trend of impact of different straw returning on LAI in three- and six-leaf stages during 2021-2023 was consistent, with T1>CK>T2, as seen in Figure 5f. The impact of treatment on LAI of wheat at the three-leaf stage reached a significant level, but the interaction effect was not significant, while the impact of year and treatment on LAI at the six-leaf stage reached a very significant level, and the interaction effect was significant. The results indicate that the growth of wheat seedlings was greatly influenced by different years, especially for their later growth and development. This may be related to large interannual temperature changes and uneven rainfall, as well as climate differences.



Note: LAR: The ratio of the total leaf area to the dry weight of the plant; RGR: The relative increase in plant biomass per unit of time; NAR: The amount of dry matter accumulation per unit leaf area per unit of time; SLA: The ratio of the leaf area to the dry weight of the leaf; LWR: The proportion of the dry weight of the leaves to the total dry weight of the plant; LAI: The ratio of the total leaf area of plants to the land area per unit of land area.

Figure 5 Effects of straw incorporation on growth rate of winter wheat seedlings from the three- to the six-leaf stage

3.7 Grain yield

As shown in Figure 6, straw incorporation increased grain yields. In 2021, the T1 and T2 treatments increased grain yields by 3.33% and 1.75%, respectively, compared with that of the CK. In 2021, the T1 and T2 treatments increased grain yields by 3.86% and 13.13%, respectively, compared with that of the CK. During the two growing seasons, the T1 and T2 treatments increased grain yield by a mean of 3.58% and 6.98%, respectively, compared with that of the CK. The impact of year on grain yield was extremely significant, but the impact of the treatments on yield was not significant and the interaction effect was not significant. These results indicated that straw return could improve the grain yield, but the year had an important effect on yield. Thus, yield may be strongly determined by agrometeorological conditions in different crop growing seasons.

3.8 Correlation analysis between growth indicators at the six-leaf stage and grain yield

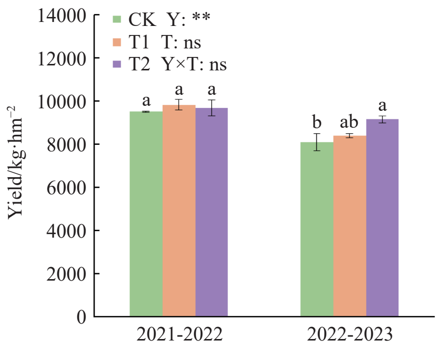
Correlation analysis between growth indicators at the six-leaf stage and grain yield revealed that grain yield had a strongly significant correlation with dry weight of leaf sheaths, tillers, number of secondary roots, and dry weight of roots, as is clear from Figure 7. This result indicated that root growth of seedlings at the six-leaf stage was an important factor that impacted the grain yield.

3.9 Principal component analysis and comprehensive evaluation based on growth analysis

Using SPSS analysis to obtain the values of main components of each indicator, the following formula was used to calculate the weighting value for each indicator:

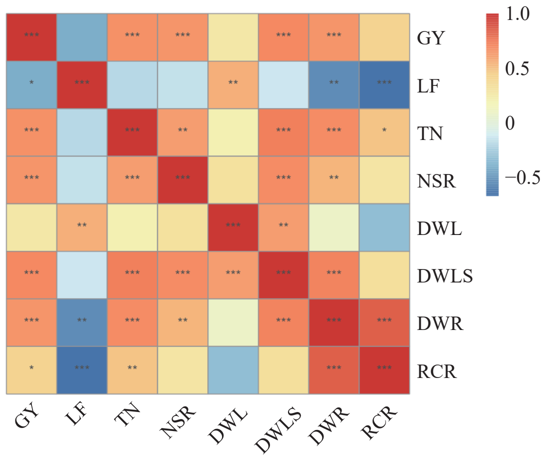
$$P_i = \frac{\lambda}{\sqrt{\mu}} \tag{8}$$

where, P_i is the weighting value, λ is the value of the main components of each indicator, and μ is the initial characteristic value (total).



Note: In the figure, means with different lowercase letters are significantly different (p<0.05), and means that share a lowercase letter are not significantly different (p>0.05). Analysis of interaction results are shown (*, **=significant at 0.05, 0.01 probability levels; ns=not significant). The "Y" refers to year; the "T" refers to treatment.

Figure 6 Effect of straw-return treatments on grain yield of wheat



Note: GY, LF, TN, NSR, DWL, DWLS, DWR, and RCR refer to grain yield, leaf area, tillers number, number of second root, dry weight of leaf, dry weight of leaf sheaths, dry weight of roots, and root-canopy ratio, respectively.

Figure 7 Correlation analysis between growth indicators at six-leaf stage and grain yield of wheat

A principal component analysis was performed on nine growth indicators associated with the growth analysis in the two wheat growing seasons from 2021 to 2023. Two principal components were extracted for each year. The cumulative contribution rates reached 100%, which represent the quality evaluation coefficients of the seedlings for the nine growth indicators, as shown in Table 6. From 2021-2022, SLA3, LAI6, and NAR were the main indicators that contributed to the first principal component, comprising 64.69% of the information content in the original data, as seen in Table 7. The main factors that contributed to the second principal component were RGR, LAI3, and LWR3, which comprised 35.31% of the information content in the original data. From 2022-2023, the main factors contributing to the first principal component were LAR, LAI6, and NAR, which comprised 68.14% of the information content in the original data. The most important factors that determined the second principal component were SLA3, LWR3, and LWR6, which comprised 31.86% of the information content of the original data. Among these indicators, NAR and LAI6 were the main factors that influenced the first component in the two years, suggesting that both indicators are particularly important for the quality of wheat growth.

Table 6 Eigenvalues and cumulative contribution of growth indicators to first and second principal components

Year	Component	Initial eigenvalue		
		Total	Percentage of variance	Cumulative/%
2021-2022	1	5.822	64.686	64.686
	2	3.178	35.314	100.000
2022-2023	1	6.133	68.140	68.140
	2	2.867	31.860	100.000

Year	Component	Extract the sum of squared loads		
		Total	Percentage of variance	Cumulative/%
2021-2022	1	5.822	64.686	64.686
	2	3.178	35.314	100.000
2022-2023	1	6.133	68.140	68.140
	2	2.867	31.860	100.000

Table 7 Principal component values and weighting values

Index	2021-2022			
	Principal component value		Weight value	
	1	2	1	2
NAR	0.845	−0.535	0.350	−0.300
LWR6	−0.730	0.683	−0.303	0.383
LAI3	0.680	−0.734	0.282	−0.411
LWR3	−0.699	0.715	−0.290	0.401
LAI6	0.948	0.319	0.393	0.179
SLA6	0.832	0.554	0.345	0.311
LAR	0.838	0.546	0.347	0.306
SLA3	0.969	0.247	0.402	0.139
RGR	0.626	0.780	0.259	0.438

Index	2022-2023			
	Principal component value		Weight value	
	1	2	1	2
NAR	0.980	0.201	0.396	0.119
LWR6	−0.717	−0.697	−0.290	−0.411
LAI3	0.968	−0.251	0.391	−0.148
LWR3	−0.245	0.970	−0.099	0.573
LAI6	0.991	0.137	0.400	0.081
SLA6	−0.840	0.543	−0.339	0.321
LAR	−0.998	−0.068	−0.403	−0.040
SLA3	0.238	−0.971	0.096	−0.574
RGR	0.961	0.277	0.388	0.164

Note: SLA3 is the specific leaf area at the three-leaf stage, SLA6 is the specific leaf area at the six-leaf stage, LWR3 is the leaf weight ratio at the three-leaf stage, and LWR6 is the leaf weight ratio at the six-leaf stage.

4 Discussion

4.1 Effects of straw incorporation on seedling emergence and emergence rate

The rapid emergence and establishment of wheat seedlings are mainly related to soil compaction, water content, temperature, etc.[17]. Zuo et al.[18] reported that, under rice straw return to the field, shallow sowing and compaction significantly increase soil moisture and increase the emergence rate of dryland rapeseed. Yin et al.[19] pointed out that, in arid areas, the no-tillage method with straw mulching can effectively moderate soil temperature changes and increase wheat production. However, some studies have also indicated that straw returning can reduce soil temperature[20]. Therefore, the impact of straw returning on soil temperature should be affected by the straw-returning method and geographical factors. In the present study, the SET and the SEPD of winter wheat seedlings were delayed compared with those of the CK, and the SES was significantly lower than that of the CK. This may be because after straw returning, the soil porosity increased[21], the seeds were unevenly distributed in the soil, and at the same time, the gas-water exchange rate accelerated, resulting in drastic changes in soil temperature and moisture. This affected the breaking of wheat dormancy[22] and the decomposition and transformation of internal storage substances in the seeds during the germination process[23], thus reducing the emergence speed and delaying the arrival of the emergence peak, but it did not affect the final emergence rate. In the 2021-2022 wheat growing season, the rainfall during the suitable sowing period promoted the rapid emergence of wheat. However, due to the poor water-holding capacity of lime concretion black soil[24] and the increased soil porosity[21] after the increase in straw returning amount, the rapid gas-water exchange led to rapid water loss, which was not conducive to the root growth after emergence. As a result, the emergence rate of treatment T1 was higher than that of T2 in that year, but the final emergence rate was lower than that of T2.

4.2 Effects of straw incorporation on seedling quality and grain yield

The formation of strong wheat seedlings is the result of coordinated development between the aboveground and underground parts of the plant. An adequate number of large tillers and robust roots are essential[25]. Previous studies have pointed out that under long-term straw return, rotary tillage with drill sowing significantly increases the tiller number, leaf area, and root weight of seedlings, thus enhancing the growth potential of individual wheat plants[26]. Under the conditions of this experiment, the number of the first large tillers and secondary roots of winter wheat at the six-leaf stage reached three or more and seven or more, respectively (Table 3 and 4), and the number of primary large tillers in the 2021-2022 wheat growing season was significantly higher than that of CK. This may be because the good soil moisture content during the suitable sowing period promoted the rapid emergence of wheat. Although the emergence rate decreased, long-term straw return promoted an increase in available nutrients and organic matter[27], and also reduced nutrient runoff[28]. Based on the nutrient compensation effect[29], the individual wheat plants had sufficient nutrient supply, which promoted the synchronous development of tillers, roots, and biomass[30]. The developed root system provided more nitrogen for the plants[31-32], thus laying a solid foundation for increasing the rate of tillers developing into ears in the subsequent growth process. This also explains why the grain yields of the straw-returning treatments were higher than that of the CK in this study. However, in the 2022-2023 wheat growing season, the grain yield of T2 was higher than that of T1 (Figure 6, $p<0.05$). It is analyzed that this may be because there was little rainfall during the vigorous root-growth period, and drought can promote the rooting of roots downward, laying a foundation for the absorption and transportation of nutrients in the later stage, thus promoting the formation of yield[33].

4.3 Principal component analysis for seedling quality evaluation

A concise and efficient method for judging the condition of wheat seedlings is conducive to assessing the pre-winter condition of wheat seedlings, providing technical support to promote the growth of strong and uniform wheat seedlings. Principal component analysis is a statistical method that converts multiple variables into a small number of principal components through a dimensionality reduction technique. Multiple indicators can be used to evaluate and rank different treatments, which can better reflect the quality of treatments[34,35]. The present study was based on nine indicators associated with wheat seedling growth, and principal component analysis was used to extract two principal components. Among these indicators, the main determining factors for the first principal component were the photosynthate production factors LAI6 and NAR. The main factor that determined the second principal component was the dry-matter accumulation factor LWR3. This indicates that the main factor influencing the development of strong seedlings in wheat is the net photosynthetic assimilation rate of the seedlings, whereas photosynthate accumulation in the leaves is a secondary influencing factor. After entering the three-leaf stage, nutrient acquisition changes from heterotrophic to autotrophic. The increase in wheat leaf area after the three-leaf stage can promote photosynthate production, improve the net assimilation rate, and achieve dry-matter accumulation, thus promoting strong seedling development. This provides a solution to the poor quality of wheat seedlings at the three-leaf stage, which can be promoted by applying a tiller fertilizer or spraying with a foliar fertilizer to increase the development of tillers, increase the green leaf area, improve photosynthate production, and ultimately achieve strong seedlings for overwintering.

5 Conclusions

Robust seedlings are essential to ensure higher photosynthate production, dry-matter accumulation, and higher yield in wheat. The negative effect of long-term straw incorporation on wheat seedling emergence was reflected in the delays in the SET and SEPD, and the decrease in the SES, but had no significant effect on the emergence rate. For this reason, the wheat seedling quality was poor when entering the three-leaf stage, but with the prolonged duration of straw return to the field and straw decomposition, the quality of wheat seedlings was significantly improved at the six-leaf stage. These findings provide theoretical support for the improvement of seedling emergence and the development of strong seedlings with straw return to the field.

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