

Article

Regulatory Effects of Basic Seedling Density on Photosynthetic Characteristics, Root Morphology and Yield of Late-Sown Wheat Under Different Soil Types

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Abstract

Soil types vary considerably and sowing dates are generally delayed for wheat production in the Yangtze–Huaihe River Basin. To identify the differential yield responses to increased basic seedling density in late-sown wheat under different soil types, this study investigated the interactive effects of soil type (sandy loam vs. clay soil) and basic seedling density (2.8×10^6 and 4.5×10^6 grains hm^{-2}) on yield formation, canopy photosynthesis, root morphology, and nitrogen utilization enzymes in late-sown wheat (cv. Yangmai 25). Increasing seedling density enhanced effective spike number, grain number per spike, tiller dynamics, leaf area index, aboveground dry matter accumulation, root activity, and grain yield; however, these increases were statistically significant only under sandy soil conditions. At the same density, sandy soil exhibited higher SPAD values, net photosynthetic rate, photosynthetic potential, and root morphological traits than clay soil. Although clay soil increased nitrogen utilization enzyme activities (NR, NiR, GS, and GOGAT) at heading and mid-filling stages, this advantage could not offset the yield disadvantages caused by restricted photosynthetic capacity and root development. Overall, soil type-dependent responses were detected for yield components, tiller traits, physiological characteristics and root morphological, indicating that soil conditions may influence the mechanisms through which density affects late-sown wheat.

Keywords: wheat (*Triticum aestivum* L.); yield formation; canopy photosynthesis; root activity; nitrogen utilization enzyme



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1. Introduction

Wheat (*Triticum aestivum* L.) is one of the major food crops in China. Stable grain yield and improved quality of wheat are essential for meeting agricultural production and market demands, as well as safeguarding national food security [1]. Since japonica rice has a longer growth period than indica rice, the implementation of the “indica-to-japonica rice conversion” project has postponed the rice harvesting period, which further delays

the subsequent wheat crop from being sown at the traditional optimal time [2,3]. Delayed wheat sowing is widespread in the rice–wheat rotation regions of the Yangtze–Huaihe River Basin [4], and the area of late-sown wheat accounts for over 40% of the total wheat area in Jiangsu Province [5].

In the Yangtze–Huaihe River Basin, late sowing generally refers to sowing conducted in mid-November or later. At this time, the accumulated temperature before winter decreases markedly, the tillering stage is shortened, individual plant biomass accumulation is insufficient, and the formation of effective spikes is inhibited [6,7]. Meanwhile, the compressed growth period narrows the time window for root development and shortens the photosynthetic functional duration. The proportion of dry matter accumulation after anthesis also declines, which further restricts the yield potential [8,9]. A one-day delay in the sowing date of winter wheat causes a yield loss of 0.35% to 1.00%, and the specific value varies with wheat cultivar, location, and climatic conditions [6,10].

Yield formation of wheat results from the synergistic effects of aboveground photosynthesis, dry matter accumulation and distribution, as well as nutrient uptake and utilization by roots [11]. As a fundamental environmental factor in farmland ecosystems, soil type not only directly regulates root morphology formation, but also indirectly affects aboveground photosynthetic characteristics and population structure by modifying the canopy microenvironment [12]. Root morphology and photosynthetic characteristics are tightly coupled, as root-derived water and nutrient supply determines leaf gas exchange capacity and shoot biomass accumulation [13]. It is therefore a key factor restricting wheat growth, development, and yield formation [12–14]. Basic seedling density regulates plant density, thereby affecting tiller dynamics, spike formation rate, and resource competition between individuals and populations. Higher density increases canopy photosynthetic area and spike number per unit area, but intensifies interplant competition for light and nutrients, reducing single-leaf photosynthetic rate and kernel number per spike. It further interacts with soil type to jointly shape the growth process and yield components of wheat [15,16].

Numerous studies have explored the separate effects of soil type or basic seedling density on wheat growth [17–20], yet most investigations have focused on only a single factor. However, the interactive effects of two typical soil types (sandy soil and clay soil) coupled with varying basic seedling densities on dynamic responses of photosynthetic characteristics, root morphology formation, and nitrogen utilization enzyme activities in wheat remain unclear. In addition, how to reconcile the conflicts between individual and population traits of wheat has not been well elucidated, especially lacking support from field experiments under late-sown conditions in the humid subtropical climate of the Yangtze–Huaihe River Basin.

As one of China's major wheat-producing regions, the Yangtze–Huaihe River Basin features diverse soil types with widespread distribution of sandy and clay soils [21]. Benefiting from distinctive matching of light, temperature, and precipitation resources, this region experiences increasingly prevalent late-sown wheat. Exploring the interactive effects of soil type and basic seedling density under late-sown conditions has important practical value for optimizing local wheat cultivation techniques.

To address the aforementioned knowledge gaps, this study was designed to investigate the interactive effects of soil type and basic seedling density on yield formation, photosynthetic characteristics, root morphology, and nitrogen utilization in late-sown wheat. Specifically, this study aimed to (1) identify the differential yield responses to increased basic seedling density in late-sown wheat under different soil types; (2) clarify the regulatory effects of the soil type–density interaction on canopy photosynthetic characteristics and dry matter accumulation; and (3) characterize the response patterns of root morphology development and nitrogen utilization enzyme activities to this interaction. Based on the above

results, this work seeks to provide a theoretical basis for precision density management of late-sown wheat based on soil type in the Yangtze–Huaihe River Basin.

2. Materials and Methods

2.1. Experimental Site and Plant Material

The study was conducted over two growing seasons (2023–2024 and 2024–2025) at the Experimental Base of the College of Agriculture, Yangzhou University, Yangzhou, Jiangsu Province (32°29′17.2″ N, 119°12′27.0″ E). One set of bins was filled with sandy soil (sand 66.42%, silt 24.71%, clay 8.87%, USDA: sandy loam), and the other with clay soil (sand 21.33%, silt 34.62%, clay 44.05%, USDA: clay). The physicochemical properties of topsoil (0–20 cm) before wheat sowing are listed in Table 1. The tested wheat cultivar was Yangmai 25, supplied by the Lixiahe Institute of Agricultural Sciences, Jiangsu Province. The study site is located in a region of humid subtropical monsoon climate. The mean, maximum, and minimum temperature, and rainfall of the study area during the overwintering, jointing, booting, anthesis and maturity stages of wheat in 2023 and 2025 are listed in Table 2. To prevent waterlogging, a drainage ditch (15 cm in width and depth) was centrally installed in each plot to facilitate surface water runoff during heavy precipitation events.

Table 1. Physicochemical properties of 0–20 cm topsoil before wheat sowing.

Years	Soil Type	Total Nitrogen (g kg ^{−1})	Organic Matter (g kg ^{−1})	Available Phosphorus (mg kg ^{−1})	Available Potassium (mg kg ^{−1})
2023	Sandy soil	1.54	11.58	31.19	46.82
	Clay soil	1.77	12.95	27.13	88.73
2024	Sandy soil	1.46	12.04	38.82	54.94
	Clay soil	1.63	13.52	36.46	98.19

Table 2. Maximum (T_{max}), minimum (T_{min}), and mean (T_{mean}) temperature, rainfall (prec) of the study area in different growth stages of wheat in 2023 and 2025.

Years	Period	T _{max} (°C)	T _{min} (°C)	T _{mean} (°C)	Prec (mm)
2023–2024	overwintering ^a	13.71	4.47	8.72	137.69
	jointing ^b	10.25	1.83	5.81	118.83
	booting ^c	19.04	8.76	13.75	23.67
	anthesis ^d	23.87	13.74	18.63	33.61
	maturity ^e	24.62	14.31	19.48	14.93
2024–2025	overwintering ^a	10.53	2.64	6.41	7.67
	jointing ^b	11.86	2.47	6.94	23.78
	booting ^c	18.42	7.43	12.67	7.94
	anthesis ^d	23.45	9.82	16.39	34.15
	maturity ^e	27.68	15.43	21.45	95.65

^a From sowing to overwintering stage. From 20 November to 14 December in 2023–2024 and 23 November to 24 December in 2024–2025. ^b From overwintering end to jointing stage. From 15 December to 18 March in 2023–2024 and 25 December to 12 March in 2024–2025. ^c From jointing end to booting stage. From 19 March to 15 April in 2023–2024 and 13 March to 1 April in 2024–2025. ^d From booting end to anthesis stage. From 16 April to 25 April in 2023–2024 and 2 April to 13 April in 2024–2025. ^e From anthesis end to maturity stage. From 26 April to 17 May in 2023–2024 and 14 April to 21 May in 2024–2025.

2.2. Experimental Design and Field Management

A two-factor split-plot design was used in this trial, with soil type as the main plot factor and basic seedling density as the subplot factor. Two levels were set for soil type (sandy soil and clay soil), and two levels for seedling density (2.8 × 10⁶ grains hm^{−2} and

4.5×10^6 grains hm^{-2}). The four treatments (Table 3) were arranged in randomized blocks within each growing season, with three replications per treatment per year. Schematic diagram of experimental layout for all treatments (Figure 1). Each experimental unit was a cement bin ($1.8 \text{ m} \times 1.7 \text{ m} \times 1.2 \text{ m}$ depth, 3.06 m^2 surface area). Sowing was performed via hill-dropping at a row and plant spacing of $25 \text{ cm} \times 11.4 \text{ cm}$, with density levels set by adjusting seed quantity per hill (8 or 13 seeds per hill). Wheat was sown on 20 November and harvested on 18 May in the 2023–2024, while it was sown on 23 November and harvested on 22 May for the 2024–2025. Nitrogen fertilizer was applied at a rate of 240 kg hm^{-2} pure N, with a basal and tillering fertilizer to booting fertilizer ratio of 6:4. Field water management, pest, disease, and weed control, as well as other agronomic practices, were implemented following high-yield cultivation protocols.

Table 3. Experimental design.

Treatment	Soil Type	Basic Seedling Density (Grains hm^{-2})
SD1	Sandy soil	2.8×10^6
SD2	Sandy soil	4.5×10^6
CD1	Clay soil	2.8×10^6
CD2	Clay soil	4.5×10^6

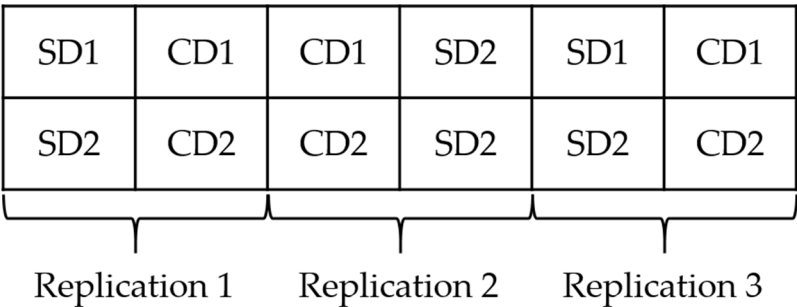


Figure 1. Schematic diagram of experimental layout for all treatments. Note: SD1, sandy soil + low basic seedling density (2.8×10^6 grains hm^{-2}); SD2, sandy soil + high basic seedling density (4.5×10^6 grains hm^{-2}); CD1, clay soil + low basic seedling density (2.8×10^6 grains hm^{-2}); CD2, clay soil + high basic seedling density (4.5×10^6 grains hm^{-2}).

2.3. Measurements

2.3.1. Yield and Its Components

At the wheat maturity (180 days after sowing in 2024 and 2025) stage, three 1m long rows were randomly selected from each plot to count spike number (spikes with ≥ 5 grains were defined as effective spikes), and the effective spike number was subsequently calculated. Three hills were sampled according to the average spike number per hill to determine grains per spike and 1000-kernel weight. Grain yield was determined by harvesting and threshing plants from a 1 m^2 area plus 50 consecutive hills in each plot, with three replicates per treatment. After air-drying, grain yield was adjusted to a standard moisture content of 13%.

2.3.2. Tiller Dynamics

At the overwintering (25 and 32 days after sowing in 2024 and 2025, jointing (120 and 110 days after sowing in 2024 and 2025), booting (148 and 130 days after sowing in 2024 and 2025), and maturity stages, the number of wheat tillers per 1 m^2 was recorded for each plot. Three replications were selected for data collection, and the collected data

were then converted to the number of tillers per unit area. Additionally, the percentage of spike-bearing tillers was calculated.

The relevant calculation formulas are as follows:

$$\text{Spike-bearing tiller percentage (\%)} = \frac{\text{Final number of effective spikes}}{\text{Maximum total number of tillers}} \times 100\% \quad (1)$$

2.3.3. Photosynthetic Characteristics

At the heading (158 and 142 days after sowing in 2024 and 2025) and mid-filling (170 and 154 days after sowing in 2024 and 2025) stages, measurements were conducted on sunny days between 09:00 and 11:00. From each plot, ten flag leaves on main stems were selected from plants with uniform height and growth status. A LI-6800 portable photosynthetic system (LI-COR, Lincoln, NE, USA) was used to determine the net photosynthetic rate (P_n), stomatal conductance (G_s), transpiration rate (T_r), and intercellular CO_2 concentration (C_i) of the flag leaves.

2.3.4. Dry Matter Accumulation and Leaf Area Index (LAI)

Leaf area and dry matter accumulation were determined at the overwintering, jointing, booting, anthesis (158 and 142 days after sowing in 2024 and 2025), and maturity stages. Three representative plants per plot were sampled based on average tiller number; roots were discarded, and all leaves were separated for leaf area measurement using an automatic leaf area meter. The sampled plants were heated at 105 °C for 30 min to deactivate enzymes, followed by oven-drying at 80 °C for four days until constant weight, then weighed. Dry matter accumulation amount and allocation ratio in each growth stage were calculated accordingly.

The relevant calculation formulas are as follows:

$$\text{Accumulation ratio (\%)} = \frac{\text{Staged dry matter accumulation}}{\text{Total dry matter accumulation}} \times 100\% \quad (2)$$

$$\text{Leaf area duration (LAD)} = \text{LAI} \times \text{Time interval} \quad (3)$$

$$\text{Canopy photosynthetic rate} = \text{LAI} \times P_n \quad (4)$$

2.3.5. Flag Leaf Relative Chlorophyll Content (Represented by SPAD Value)

At the heading and mid-filling stages, 10 flag leaves with consistent orientation and growth status were selected from each plot. The relative chlorophyll content in the middle part of each flag leaf was measured using an SPAD-502 chlorophyll meter (Konica Minolta, Inc., Tokyo, Japan), with 10 replications per treatment.

2.3.6. Leaf Photosynthetic Enzymes

At the heading and mid-filling stages, uniformly growing plants were selected for sampling. Wheat flag leaves were collected, immediately frozen in liquid nitrogen, and stored at −80 °C for subsequent determinations. The rubisco activity was measured using a commercial microassay kit provided by Suzhou Comin Biotechnology Co., Ltd., Suzhou, China.

2.3.7. Root Morphology

At the overwintering, regreening (80 and 75 days after sowing in 2024 and 2025), and jointing stages, samples were collected from each treatment based on the average tiller number. Soil blocks with a depth of 30 cm containing intact root systems were excavated. The soil blocks were rinsed with running water to remove surface soil, then soaked in nylon mesh bags to obtain complete root systems. Root morphology parameters were scanned

using an Epson Expression 10000XL scanner (Seiko Epson Corporation, Suwa, Nagano, Japan) and analyzed with WinRHIZO 2025a software. Root morphology indices per unit area were calculated.

2.3.8. Root Activity

Three representative plants with uniform growth were sampled per treatment at the overwintering, regreening, and jointing stages based on average tiller number. Intact root samples were excavated from the 0–30 cm soil layer and gently rinsed with running water to remove all adhered soil particles. Root activity was determined using the α -naphthylamine oxidation method.

2.3.9. Nitrogen Utilization Enzymes in Roots

Uniform wheat plants were sampled at heading and mid-filling stages, and 0.5 g fresh root tissue was weighed for enzyme extraction. Samples were homogenized in an ice bath with 5 mL pre-cooled extraction buffer containing 50 mmol L⁻¹ Tris-HCl (pH 7.5), 1 mmol L⁻¹ EDTA, 10 mmol L⁻¹ MgCl₂, 5 mmol L⁻¹ DTT, and 1% PVP. The homogenate was centrifuged at 12,000× *g* and 4 °C for 20 min, and the resulting supernatant was collected as crude enzyme extract to assay the activities of nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS), and glutamate synthase (GOGAT). NR activity was determined by the in vitro sulfanilamide colorimetric method [22]; NiR by an in vitro colorimetric assay [23]; GS by the γ -glutamylhydroxamate synthesis method [24]; and GOGAT by the NADH oxidation spectrophotometric method [25,26].

2.4. Data Analysis

Analysis of variance (ANOVA, $p < 0.05$) was adopted to analyze the effects of experimental year, soil type, basic seedling density, and their interactions on all measured indicators. Prior to ANOVA, all data were tested for normality (Shapiro–Wilk test, $p > 0.05$) and homogeneity of variance (Levene’s test, $p > 0.05$). Data with unequal variances were log ($x + 1$) transformed to meet the assumption of homogeneity of variance. The Least Significant Difference (LSD) test ($p < 0.05$) was applied for multiple comparisons among treatment means. All statistical analyses were performed using SPSS 20 software (SPSS, Chicago, IL, USA) and figures were generated using OriginPro 2026b software (OriginLab, Hampton, MA, USA).

3. Results

3.1. Wheat Yield and Yield Components as Affected by Soil Type and Basic Seedling Density

Soil type and basic seedling density both significantly affected grain yield and yield components of wheat, although, the interactive effects of soil type and basic seedling density were significant only for grains per spike and effective spike number (Table 4). Under sandy soil conditions, grain yield increased significantly with rising basic seedling density, and the two-year average grain yield of SD2 was 5.64% higher than that of SD1. Under clay soil conditions, grain yield slightly increased with increased basic seedling density; the average grain yield of CD2 was enhanced by 2.82% relative to CD1, yet the difference was statistically non-significant. Under D1 treatment, grain yield differed significantly between soil types; the two-year average grain yield in sandy soil was significantly increased by 6.52% compared with clay soil. Under D2 treatment, soil type also exerted a significant effect on grain yield, with sandy soil yielding 9.44% higher on average across two years relative to clay soil.

Table 4. Effects of soil type and basic seedling density on yield and its components.

Years	Treatment	Grains Per Spike	1000-Kernel Weight (g)	Effective Spikes ($\times 10^4 \text{ hm}^{-2}$)	Grain Yield (t hm^{-2})
2024	SD1	38.72 b	41.97 a	490.25 b	7.62 b
	SD2	41.89 a	39.05 b	503.98 a	8.03 a
	CD1	37.61 b	38.94 b	478.83 c	7.09 c
	CD2	38.82 b	37.46 c	487.71 bc	7.25 c
2025	SD1	37.56 b	43.35 a	465.64 b	7.09 b
	SD2	39.88 a	41.66 bc	488.11 a	7.51 a
	CD1	36.15 c	42.83 ab	460.33 b	6.72 c
	CD2	37.20 bc	41.03 c	462.76 b	6.95 c
Year (Y)		20.52 **	85.15 **	76.34 **	12.86 **
Soil (S)		35.93 **	21.62 **	36.90 **	82.66 **
Density (D)		31.55 **	40.43 **	24.46 **	15.25 **
Y $\times$ S		ns	7.82 *	ns	ns
Y $\times$ D		ns	ns	ns	ns
S $\times$ D		5.48 *	ns	6.71 *	ns
Y $\times$ S $\times$ D		ns	ns	ns	ns

Note: SD1, sandy soil + low basic seedling density (2.8×10^6 grains hm^{-2}); SD2, sandy soil + high basic seedling density (4.5×10^6 grains hm^{-2}); CD1, clay soil + low basic seedling density (2.8×10^6 grains hm^{-2}); CD2, clay soil + high basic seedling density (4.5×10^6 grains hm^{-2}). Different letters indicated significant difference between treatments at the 0.05 probability level. * and ** indicate significant differences among levels within a factor at the 0.05 and 0.01 levels, respectively; ns indicates no significant difference.

As shown in Table 4, increasing basic seedling density exerted divergent effects on the number of effective spikes and grains per spike under the two soil types. Under sandy soil conditions, the two-year average number of effective spikes of SD2 was increased by 3.82% relative to SD1. Under clay soil conditions, the corresponding increment for CD2 versus CD1 was only 1.19%. Increasing basic seedling density produced consistent effects on grains per spike across the two soil types. The two-year average grains per spike of SD2 was 7.19% higher than SD1 under sandy soil conditions, while CD2 increased by 3.06% relative to CD1 under clay soil conditions. By contrast, higher basic seedling density negatively affected 1000-kernel weight in both soils: compared with SD1, SD2 reduced average 1000-kernel weight by 5.43% under sandy soil conditions, and CD2 decreased this index by 4.00% relative to CD1 under clay soil conditions. At the identical basic seedling density, soil type significantly affected the number of effective spikes. Relative to clay soil, sandy soil increased the two-year average effective spike number by 1.77% under SD1 and by 4.41% under SD2.

3.2. Responses of Wheat Tiller Dynamics and Spike-Bearing Tiller Percentage to Soil Type and Basic Seedling Density

Soil type and basic seedling density significantly affected tiller dynamics and spike-bearing tiller percentage of wheat, and soil type and basic seedling density exerted significant interactive effects on tiller number at all growth stages except the overwintering stage, as well as on productive tiller percentage (Table 5). Under identical soil type, tiller number increased with the rise in basic seedling density. Under sandy soil conditions, the two-year average tiller numbers of SD2 were increased by 4.76%, 7.00%, 5.34%, and 10.56% compared with SD1 at overwintering, jointing, booting, and maturity stages, respectively. Under clay soil conditions, CD2 showed respective increases of 7.01%, 2.39%, 3.05%, and 3.27% relative to CD1 across the four growth stages.

Table 5. Effects of soil type and basic seedling density on wheat tiller dynamics and spike-bearing tiller percentage.

Years	Treatment	Overwintering ($\times 10^4 \text{ hm}^{-2}$)	Jointing ($\times 10^4 \text{ hm}^{-2}$)	Booting ($\times 10^4 \text{ hm}^{-2}$)	Maturity ($\times 10^4 \text{ hm}^{-2}$)	Spike Bearing Tiller Percentage (%)
2024	SD1	334.59 b	914.63 b	556.53 b	395.33 b	0.43 b
	SD2	351.37 a	998.45 a	604.27 a	452.58 a	0.45 a
	CD1	308.96 c	907.61 b	518.16 c	386.28 c	0.42 c
	CD2	337.30 b	916.34 b	527.26 c	390.66 bc	0.43 bc
2025	SD1	331.96 ab	912.22 b	568.92 b	411.59 b	0.45 b
	SD2	346.93 a	956.30 a	596.00 a	448.20 a	0.47 a
	CD1	309.28 c	874.16 c	516.28 c	369.25 c	0.42 d
	CD2	324.29 bc	908.22 bc	538.66 c	389.30 bc	0.43 c
Year (Y)		ns	17.12 **	ns	ns	40.81 **
Soil (S)		51.27 **	70.89 **	208.70 **	286.35 **	291.60 **
Density (D)		40.00 **	67.24 **	46.43 **	127.2 **	98.57 **
Y $\times$ S		ns	ns	ns	9.44 **	26.04 **
Y $\times$ D		ns	ns	ns	0.56	ns
S $\times$ D		ns	16.72 **	7.72 *	60.28 **	22.64 **
Y $\times$ S $\times$ D		ns	9.77 **	4.73 *	6.52 *	ns

Note: SD1, sandy soil + low basic seedling density (2.8×10^6 grains hm^{-2}); SD2, sandy soil + high basic seedling density (4.5×10^6 grains hm^{-2}); CD1, clay soil + low basic seedling density (2.8×10^6 grains hm^{-2}); CD2, clay soil + high basic seedling density (4.5×10^6 grains hm^{-2}). Different letters indicated significant difference between treatments at the 0.05 probability level. * and ** indicate significant differences among levels within a factor at the 0.05 and 0.01 levels, respectively; ns indicates no significant difference.

As shown in Table 5, spike-bearing tiller percentage increased significantly with elevated basic seedling density under sandy soil conditions; the two-year average spike-bearing tiller percentage of SD2 was significantly higher than SD1 by 4.55%. Under clay soil conditions, spike-bearing tiller percentage slightly rose with increased basic seedling density. The two-year average spike-bearing tiller percentage of CD2 was 2.38% higher than CD1, with no significant difference observed between treatments. At the same basic seedling density, spike-bearing tiller percentage differed significantly among soil types. Compared with clay soil, sandy soil significantly elevated the two-year average spike-bearing tiller percentage by 4.76% under D1 and by 6.98% under D2.

3.3. Effects of Soil Type and Basic Seedling Density on Wheat Dry Matter Accumulation and Stage Accumulation

Soil type and basic seedling density both exerted significant effects on aboveground dry matter accumulation of wheat (Figure 2). Under identical soil type, aboveground dry matter accumulation was significantly enhanced with increasing basic seedling density at overwintering, jointing, booting, anthesis, and maturity stages. Under sandy soil conditions, the two-year average aboveground dry matter accumulation of D2 increased by 8.19%, 12.58%, 16.43%, 9.00%, and 6.68% relative to D1 across successive growth stages. Under clay soil conditions, the corresponding increases were 11.95%, 11.11%, 10.46%, 2.10%, and 2.73%, respectively. At the same basic seedling density, aboveground dry matter accumulation differed significantly between soil types at all sampling stages. Under D1, sandy soil increased the two-year average dry weight by 7.55%, 5.11%, 5.00%, 6.24%, and 7.62% compared with clay soil; under D2, the respective increments reached 3.93%, 6.51%, 10.68%, 13.42%, and 11.76%.

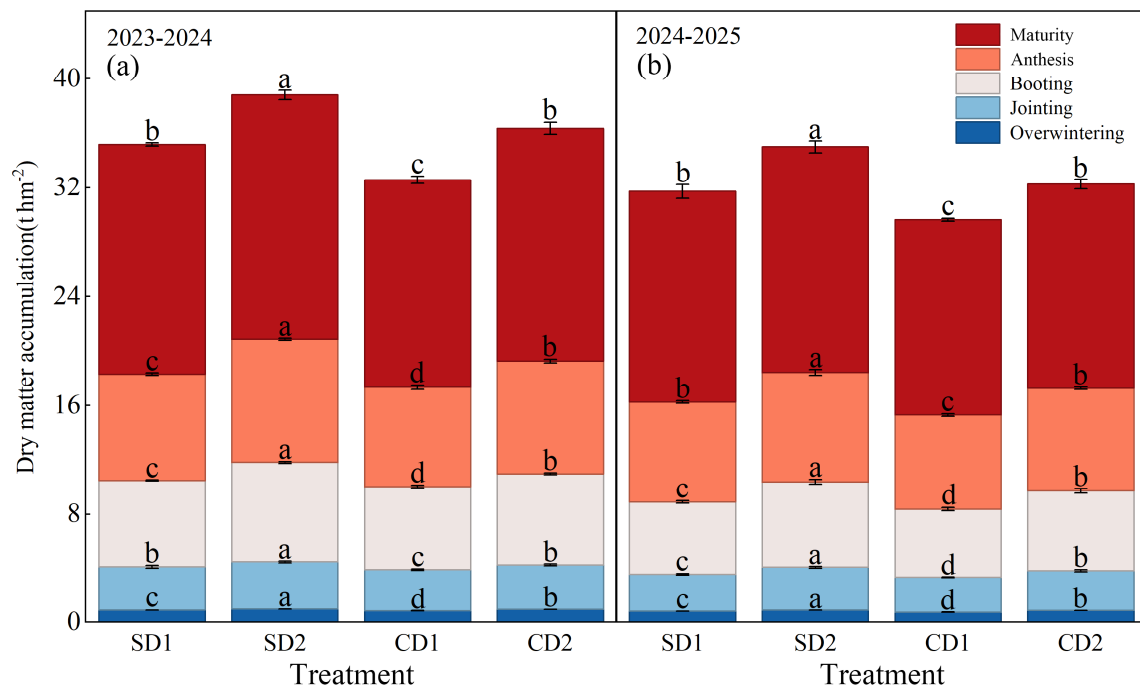


Figure 2. Effects of soil type and basic seedling density on aboveground dry matter accumulation in wheat. Note: (a) 2023–2024 growing season; (b) 2024–2025 growing season. SD1, sandy soil + low basic seedling density (2.8×10^6 grains hm^{-2}); SD2, sandy soil + high basic seedling density (4.5×10^6 grains hm^{-2}); CD1, clay soil + low basic seedling density (2.8×10^6 grains hm^{-2}); CD2, clay soil + high basic seedling density (4.5×10^6 grains hm^{-2}). Different lowercase letters indicate significant differences among treatments at the same growth stage at $p < 0.05$. Error bars represent standard deviation ($n = 3$).

Soil type and basic seedling density significantly affected stage-specific aboveground dry matter accumulation of wheat, while their significant interaction effects were not detected (Table 6). Under identical soil type, the two-year average proportion of dry matter accumulation during the overwintering–jointing stage was increased by 7.18% and 7.80% for D2 relative to D1; during the jointing–booting stage, D2 rose by 24.46% and 7.80% compared with D1, respectively. During the booting–anthesis stage, the proportion of dry matter accumulation was markedly lower in D2 relative to D1. In 2024, the reduction reached 11.60% (sandy soil) and 28.57% (clay soil); in 2025, corresponding declines were 20.48% and 23.06%, respectively. For the anthesis–maturity period: under sandy soil conditions, D2 increased by 3.92% in 2024 but declined by 1.75% in 2025; under clay soil conditions, D2 rose by 3.44% and 0.15% in the two experimental years, respectively.

Under D1 treatment, compared with clay soil, sandy soil decreased the two-year average proportion of dry matter accumulation by 3.16% at overwintering–jointing and by 18.55% at jointing–booting, whereas increments of 10.59% and 8.02% were observed at booting–anthesis and anthesis–maturity stages, respectively. Under D2, sandy soil increased the proportion of dry matter accumulation by 11.33% during overwintering–jointing relative to clay soil, whereas a reduction of 22.99% occurred at jointing–booting. In contrast, increments of 45.34% and 7.47% were detected at booting–anthesis and anthesis–maturity stages, respectively.

Table 6. Effects of soil type and basic seedling density on stage dry matter accumulation in wheat.

Years	Treatment	OW-J		J-B		B-A		A-M	
		Acc. (t hm ⁻²)	R. (%)	Acc. (t hm ⁻²)	R. (%)	Acc. (t hm ⁻²)	R. (%)	Acc. (t hm ⁻²)	R. (%)
2024	SD1	2.33 b	14.58	3.17 c	19.84	1.91 a	11.98	8.53 ab	53.60
	SD2	2.56 a	15.08	3.83 a	23.96	1.69 b	10.59	8.90 a	55.70
	CD1	2.21 c	14.89	2.95 d	24.48	1.67 b	10.36	7.89 c	49.43
	CD2	2.40 b	15.87	3.40 b	26.66	1.18 c	7.40	8.17 bc	51.13
2025	SD1	1.94 c	12.53	2.75 ab	14.78	1.99 a	12.89	8.11 b	52.45
	SD2	2.30 a	13.90	3.13 a	18.94	1.70 ab	10.25	8.53 a	51.53
	CD1	1.81 d	13.09	2.29 b	19.16	1.90 a	13.27	7.40 c	51.64
	CD2	2.11 b	14.27	2.66 ab	18.02	1.51 b	10.21	7.65 c	51.72
Year (Y)		361.30 **		58.84 **		14.37 **		29.69 **	
Soil (S)		69.99 **		22.87 **		35.02 **		81.43 **	
Density (D)		235.07 **		32.11 **		64.34 **		15.88 **	
Y × S		ns		ns		7.26 *		ns	
Y × D		11.69 **		ns		ns		ns	
S × D		ns		ns		ns		ns	
Y × S × D		ns		ns		ns		ns	

Note: SD1, sandy soil + low basic seedling density (2.8×10^6 grains hm⁻²); SD2, sandy soil + high basic seedling density (4.5×10^6 grains hm⁻²); CD1, clay soil + low basic seedling density (2.8×10^6 grains hm⁻²); CD2, clay soil + high basic seedling density (4.5×10^6 grains hm⁻²). OW-J, Overwintering (25 and 32 days after sowing in 2024 and 2025)-Jointing (120 and 110 days after sowing in 2024 and 2025); J-B, Jointing (120 and 110 days after sowing in 2024 and 2025)-Booting (148 and 130 days after sowing in 2024 and 2025); B-A, Booting (148 and 130 days after sowing in 2024 and 2025)-Anthesis (158 and 142 days after sowing in 2024 and 2025); A-M, Anthesis (158 and 142 days after sowing in 2024 and 2025)-Maturity (180 days after sowing in 2024 and 2025); Acc., Accumulation; R., Ratio. Different letters indicated significant difference between treatments at the 0.05 probability level. * and ** indicate significant differences among levels within a factor at the 0.05 and 0.01 levels, respectively; ns indicates no significant difference.

3.4. Effects of Soil Type and Basic Seedling Density on Leaf Area Index

Soil type and basic seedling density exhibited significant effects on LAI across all sampling stages, while their significant interactive effect was only detected at the maturity stage (Table 7). Under the same soil type, LAI increased with the elevation of basic seedling density across all growth stages. Under sandy soil conditions, the two-year average LAI of D2 was significantly increased by 8.92%, 6.91%, 4.50%, 4.85%, and 9.19% relative to D1 at overwintering, jointing, booting, anthesis, and maturity stages, respectively. Under clay soil conditions, the corresponding increases were 6.85%, 8.06%, 3.79%, 4.69%, and 1.67%.

Table 7. Effects of soil type and basic seedling density on leaf area index (LAI) in wheat.

Years	Treatment	Overwintering	Jointing	Booting	Anthesis	Maturity
2024	SD1	0.80 b	4.31 c	6.44 ab	5.35 b	2.91 b
	SD2	0.87 a	4.63 a	6.55 a	5.63 a	3.17 a
	CD1	0.75 c	4.12 d	6.10 c	5.14 c	2.72 c
	CD2	0.78 bc	4.42 b	6.21 bc	5.42 b	2.78 bc
2025	SD1	0.77 b	4.23 b	6.00 b	5.17 b	2.75 b
	SD2	0.84 a	4.50 a	6.45 a	5.40 a	3.01 a
	CD1	0.71 c	3.82 c	5.77 c	4.88 c	2.68 b
	CD2	0.78 b	4.16 b	6.11 b	5.07 b	2.71 b
Year (Y)		10.02 **	38.36 **	19.70 **	53.40 **	11.62 **
Soil (S)		58.93 **	85.56 **	33.17 **	55.52 **	56.35 **
Density (D)		53.01 **	97.87 **	21.94 **	49.96 **	23.94 **
Y × S		ns	7.93 *	ns	ns	ns
Y × D		ns	ns	6.58 *	ns	ns
S × D		ns	ns	ns	ns	11.26 **
Y × S × D		ns	ns	ns	ns	ns

Note: SD1, sandy soil + low basic seedling density (2.8×10^6 grains hm⁻²); SD2, sandy soil + high basic seedling density (4.5×10^6 grains hm⁻²); CD1, clay soil + low basic seedling density (2.8×10^6 grains hm⁻²); CD2, clay soil + high basic seedling density (4.5×10^6 grains hm⁻²). Different letters indicated significant difference between treatments at the 0.05 probability level. * and ** indicate significant differences among levels within a factor at the 0.05 and 0.01 levels, respectively; ns indicates no significant difference.

Compared with CD1, SD1 significantly elevated two-year average LAI by 7.53%, 7.56%, 4.80%, 4.99%, and 4.81% at overwintering, jointing, booting, anthesis, and maturity stages, respectively. Under D2, two-year average LAI was increased by 9.62%, 6.41%, 5.52%, 5.15%, and 12.57% under sandy soil conditions relative to clay soil conditions at overwintering, jointing, booting, anthesis, and maturity stages, respectively. In addition, SD2 achieved the maximum LAI across all growth stages.

3.5. Effects of Soil Type and Basic Seedling Density on SPAD Value and Rubisco Activity

Soil type and basic seedling density significantly affected leaf SPAD value and rubisco activity of photosynthetic enzymes in wheat throughout all sampling stages, whereas their interaction effect was only detectable on rubisco activity at the mid-grain filling stage (Table 8). Under identical soil type, leaf SPAD value increased with rising basic seedling density across all growth stages, whereas rubisco activity declined as basic seedling density increased. In terms of SPAD values, under sandy soil conditions, the two-year average SPAD of D2 was increased by 12.37% and 11.02% relative to D1 at heading and mid-filling stages, respectively. Under clay soil conditions, corresponding increments for D2 versus D1 reached 10.77% and 11.25%. Regarding rubisco activity, D2 significantly reduced the activity by 6.19% and 6.19% relative to D1 at heading and mid-filling under sandy soil conditions; the corresponding reductions were 18.94% and 18.94% under clay soil conditions.

Table 8. Effects of soil type and basic seedling density on relative chlorophyll content (represented by SPAD value) and photosynthetic enzymes (Rubisco) in wheat.

Years	Treatment	SPAD		Rubisco (nmol min ⁻¹ g ⁻¹)	
		Heading	Mid-Grain Filling	Heading	Mid-Grain Filling
2024	SD1	48.25 c	43.52 c	164.34 a	117.82 a
	SD2	55.18 a	48.72 a	139.87 c	109.80 b
	CD1	45.51 d	40.92 d	151.75 b	110.30 b
	CD2	51.82 b	45.62 b	132.22 d	93.78 c
2025	SD1	48.27 b	41.16 c	142.86 a	108.86 a
	SD2	53.28 a	45.29 a	133.43 b	102.84 b
	CD1	46.07 c	39.18 d	139.06 ab	105.01 b
	CD2	49.62 b	43.49 b	115.42 c	80.76 c
Year (Y)		ns	34.88 **	116.69 **	58.10 **
Soil (S)		39.56 **	33.59 **	62.59 **	121.34 **
Density (D)		131.50 **	125.59 **	210.32 **	148.98 **
Y × S		ns	ns	ns	ns
Y × D		6.05 *	ns	ns	ns
S × D		ns	ns	ns	35.41 **
Y × S × D		ns	ns	112.99 **	4.70 *

Note: SD1, sandy soil + low basic seedling density (2.8×10^6 grains hm^{-2}); SD2, sandy soil + high basic seedling density (4.5×10^6 grains hm^{-2}); CD1, clay soil + low basic seedling density (2.8×10^6 grains hm^{-2}); CD2, clay soil + high basic seedling density (4.5×10^6 grains hm^{-2}). Different letters indicated significant difference between treatments at the 0.05 probability level. * and ** indicate significant differences among levels within a factor at the 0.05 and 0.01 levels, respectively; ns indicates no significant difference.

For SPAD values, SD1 was 5.39% and 5.72% higher than CD1 at heading and mid-filling stages, respectively. For rubisco activity, sandy soil exceeded clay soil by 5.28% at both sampling dates. Under D2, SPAD values in sandy soil were 6.91% and 5.51% higher than those in clay soil at heading and mid-filling, with the maximum SPAD obtained under SD2. Compared with CD1, SD2 increased SPAD by 18.43% and 17.35% at the two corresponding stages, respectively. By contrast, for rubisco activity, sandy soil outperformed clay soil by 21.83% at both heading and mid-filling stages under D2; relative to CD1, rubisco activity of SD2 decreased by 1.24% on both sampling dates.

3.6. Effects of Soil Type and Basic Seedling Density on Photosynthetic Characteristic Parameters of Wheat Flag Leaf

As shown in Figures 3 and 4, both soil type and basic seedling density exerted significant effects on photosynthetic characteristics of wheat. Under identical soil type, P_n , G_s , T_r , and C_i declined with increasing basic seedling density. Under sandy soil conditions, P_n , G_s , T_r , and C_i decreased significantly with increased basic seedling density; the two-year average net photosynthetic rate under D2 was significantly reduced by 9.27% and 10.63% relative to D1. Under clay soil conditions, P_n also declined markedly with rising basic seedling density, with CD2 showing respective reductions of 9.23% and 10.86% in two-year average P_n compared with CD1. G_s , T_r , and C_i followed the same changing tendency as P_n .

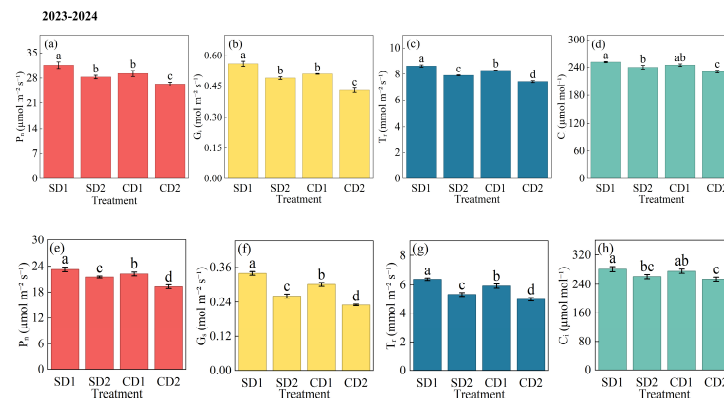


Figure 3. Effects of soil type and basic seedling density on photosynthetic characteristic parameters of wheat flag leaf in 2023–2024. Note: (a–d) Heading; (e–h) Mid-Grain Filling. (a,e) P_n , net photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$); (b,f) G_s , stomatal conductance ($\text{mol m}^{-2} \text{s}^{-1}$); (c,g) T_r , transpiration rate ($\text{mmol m}^{-2} \text{s}^{-1}$); (d,h) C_i , intercellular CO_2 concentration ($\mu\text{mol mol}^{-1}$). SD1, sandy soil + low basic seedling density (2.8×10^6 grains hm^{-2}); SD2, sandy soil + high basic seedling density (4.5×10^6 grains hm^{-2}); CD1, clay soil + low basic seedling density (2.8×10^6 grains hm^{-2}); CD2, clay soil + high basic seedling density (4.5×10^6 grains hm^{-2}). Different lowercase letters indicate significant differences among treatments at $p < 0.05$. Error bars represent standard deviation ($n = 3$).

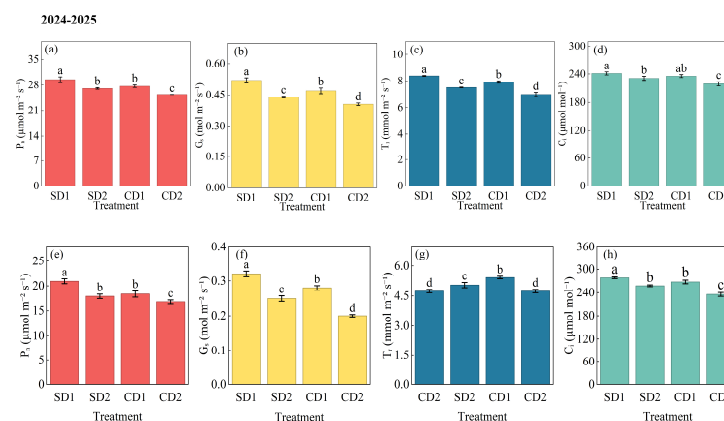


Figure 4. Effects of soil type and basic seedling density on photosynthetic characteristic parameters of wheat flag leaf in 2024–2025. Note: (a–d) Heading; (e–h) Mid-Grain Filling. (a,e) P_n , net photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$); (b,f) G_s , stomatal conductance ($\text{mol m}^{-2} \text{s}^{-1}$); (c,g) T_r , transpiration rate ($\text{mmol m}^{-2} \text{s}^{-1}$); (d,h) C_i , intercellular CO_2 concentration ($\mu\text{mol mol}^{-1}$). SD1, sandy soil + low basic seedling density (2.8×10^6 grains hm^{-2}); SD2, sandy soil + high basic seedling density (4.5×10^6 grains hm^{-2}); CD1, clay soil + low basic seedling density (2.8×10^6 grains hm^{-2}); CD2, clay soil + high basic seedling density (4.5×10^6 grains hm^{-2}). Different lowercase letters indicate significant differences among treatments at $p < 0.05$. Error bars represent standard deviation ($n = 3$).

Under the same basic seedling density, photosynthetic characteristics differed significantly among soil types. In terms of net photosynthetic rate, sandy soil increased the two-year average value by 7.15% and 9.03% relative to clay soil at heading and mid-filling, respectively. For stomatal conductance, the corresponding increments were 10.46% and 16.20%. Transpiration rate rose by 5.94% and 6.78%, while intercellular CO₂ concentration was elevated by 3.60% and 4.49% in sandy soil versus clay soil across the two stages.

3.7. Effects of Soil Type and Basic Seedling Density on Photosynthetic Potential and Canopy Photosynthetic Rate in Wheat

Soil type and basic seedling density significantly influenced wheat LAD and canopy photosynthetic rate (Table 9). However, the two factors exhibited no interactive effects on these two indicators. Significant interactive effects were only observed between year and basic seedling density for LAD. Under clay soil conditions, LAD increased with the rise in basic seedling density. Compared with D1, the two-year average LAD of D2 increased by 8.04%, 5.58%, 4.37%, and 5.78% during overwintering–jointing, jointing–booting, booting–anthesis, and anthesis–maturity stages, respectively. Under sandy soil conditions, LAD presented a continuous ascending trend as basic seedling density increased, with respective increments of 7.45%, 5.52%, 4.84%, and 6.34% for D2 versus D1 across the four growth periods above.

Table 9. Effects of soil type and basic seedling density on leaf area duration (LAD) and canopy photosynthetic rate (Canopy P_n) in wheat.

Years	Treatment	LAD (m ² d m ⁻²)				Canopy P _n (μmol CO ₂ m ⁻² s ⁻¹)
		Overwintering- Jointing	Jointing- Booting	Booting- Flowering	Flowering- Maturity	Heading
2024	SD1	250.36 c	118.25 b	53.06 b	173.46 b	150.02 a
	SD2	269.70 a	123.02 a	54.83 a	184.80 a	141.34 b
	CD1	238.73 d	112.38 c	50.57 c	164.99 c	135.82 b
	CD2	256.86 b	116.97 b	52.37 b	175.77 b	119.87 c
2025	SD1	107.41 b	117.68 b	67.06 b	229.68 b	125.68 a
	SD2	114.74 a	125.93 a	71.10 a	243.89 a	115.76 b
	CD1	97.39 c	110.32 c	63.92 c	217.21 c	106.10 c
	CD2	106.28 b	118.14 b	67.12 b	228.52 b	103.02 c
Year (Y)		26,614.23 **	ns	2361.83 **	2469.31 **	207.90 **
Soil (S)		141.10 **	135.14 **	100.92 **	104.61 **	103.25 **
Density (D)		220.53 **	119.20 **	80.95 **	115.50 **	31.62 **
Y × S		ns	ns	ns	5.44 *	ns
Y × D		34.56 **	8.32 *	9.33 **	ns	ns
S × D		ns	ns	ns	ns	ns
Y × S × D		ns	ns	ns	ns	ns

Note: SD1, sandy soil + low basic seedling density (2.8×10^6 grains hm⁻²); SD2, sandy soil + high basic seedling density (4.5×10^6 grains hm⁻²); CD1, clay soil + low basic seedling density (2.8×10^6 grains hm⁻²); CD2, clay soil + high basic seedling density (4.5×10^6 grains hm⁻²). Canopy P_n, canopy photosynthetic rate. Different letters indicated significant difference between treatments at the 0.05 probability level. * and ** indicate significant differences among levels within a factor at the 0.05 and 0.01 levels, respectively; ns indicates no significant difference.

Under identical soil type, increasing basic seedling density reduced canopy photosynthetic rate of wheat. Under sandy soil conditions, the two-year average canopy photosynthetic rate of D2 was decreased by 6.75% relative to D1, whereas the corresponding reduction reached 7.87% under clay soil conditions. At the same basic seedling density, canopy photosynthetic rate differed significantly between soil types. Under D1, the two-year average canopy photosynthetic rate under sandy soil conditions was 13.96% higher than under clay soil conditions, while the corresponding increment reached 15.35% under D2.

3.8. Effects of Soil Type and Basic Seedling Density on Root Morphology of Individual Wheat Plant

The interaction between soil type and basic seedling density significantly affected root morphology parameters of wheat across all growth stages, whereas experimental year exerted no significant influence on these root morphology traits (Figures 5 and 6). Under identical soil type, all root morphology indices decreased significantly with the increase in basic seedling density. Compared with D1, the two-year average total root length of D2 decreased by 16.48% and 18.75%, total root surface area declined by 4.63% and 11.85%, total root volume was reduced by 14.82% and 9.65%, and total root dry weight dropped by 15.05% and 15.66%, respectively.

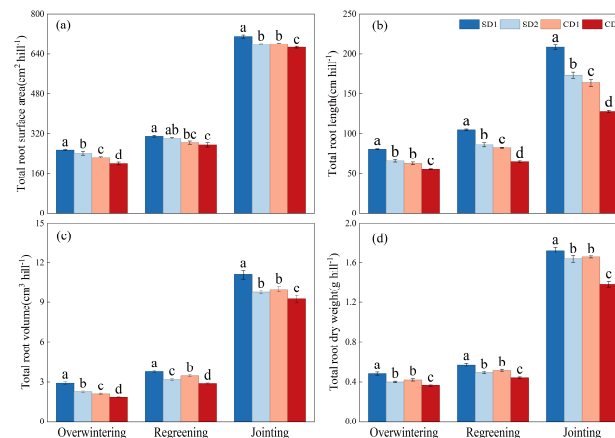


Figure 5. Effects of soil type and basic seedling density on root morphology of individual wheat plant in 2023–2024. Note: (a) total root surface area (cm² hill⁻¹); (b) total root length (cm hill⁻¹); (c) total root volume (cm³ hill⁻¹); (d) total root dry weight (g hill⁻¹). SD1, sandy soil + low basic seedling density (2.8×10^6 grains hm⁻²); SD2, sandy soil + high basic seedling density (4.5×10^6 grains hm⁻²); CD1, clay soil + low basic seedling density (2.8×10^6 grains hm⁻²); CD2, clay soil + high basic seedling density (4.5×10^6 grains hm⁻²). Different lowercase letters indicate significant differences among treatments at the same growth stage at $p < 0.05$. Error bars represent standard deviation ($n = 3$).

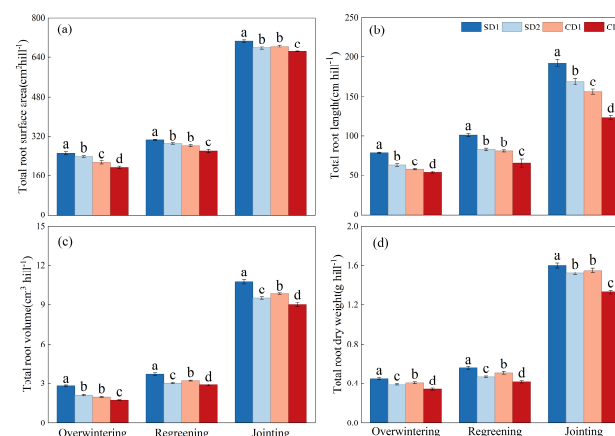


Figure 6. Effects of soil type and basic seedling density on root morphology of individual wheat plant in 2024–2025. Note: (a) total root surface area (cm² hill⁻¹); (b) total root length (cm hill⁻¹); (c) total root volume (cm³ hill⁻¹); (d) total root dry weight (g hill⁻¹). SD1, sandy soil + low basic seedling density (2.8×10^6 grains hm⁻²); SD2, sandy soil + high basic seedling density (4.5×10^6 grains hm⁻²); CD1, clay soil + low basic seedling density (2.8×10^6 grains hm⁻²); CD2, clay soil + high basic seedling density (4.5×10^6 grains hm⁻²). Different lowercase letters indicate significant differences among treatments at the same growth stage at $p < 0.05$. Error bars represent standard deviation ($n = 3$).

Under the same basic seedling density, all root morphology parameters increased significantly when soil type shifted from clay to sandy soil. Compared with clay soil, sandy soil at the identical basic seedling density increased two-year average total root length by 26.99% and 30.55%, total root surface area by 14.61% and 18.32%, total root volume by 14.59% and 7.97%, and total root dry weight by 12.05% and 12.86%, respectively.

3.9. Effects of Soil Type and Basic Seedling Density on Root Morphology per Unit Area in Wheat

Under identical soil type, root morphology indices per unit area of wheat increased significantly with rising basic seedling density (Figures 7 and 8). In each growth stage, compared with D1, the two-year average total root length per unit area of D2 increased by 50.08%, 44.02%, and 47.40% at overwintering, regreening, and jointing stages, respectively; total root surface area rose by 64.75%, 69.09%, and 70.59%; total root volume increased by 43.99%, 49.44%, and 59.13%; and total root dry weight was elevated by 50.25%, 50.21%, and 60.46%.

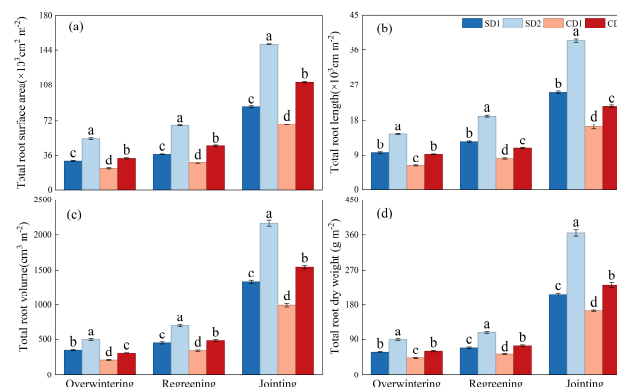


Figure 7. Effects of soil type and basic seedling density on root morphology per unit area in wheat in 2023–2024. Note: (a) total root surface area ($\text{cm}^2 \text{ m}^{-2}$); (b) total root length (cm m^{-2}); (c) total root volume ($\text{cm}^3 \text{ m}^{-2}$); (d) total root dry weight (g m^{-2}). SD1, sandy soil + low basic seedling density (2.8×10^6 grains hm^{-2}); SD2, sandy soil + high basic seedling density (4.5×10^6 grains hm^{-2}); CD1, clay soil + low basic seedling density (2.8×10^6 grains hm^{-2}); CD2, clay soil + high basic seedling density (4.5×10^6 grains hm^{-2}). Different lowercase letters indicate significant differences among treatments at the same growth stage at $p < 0.05$. Error bars represent standard deviation ($n = 3$).

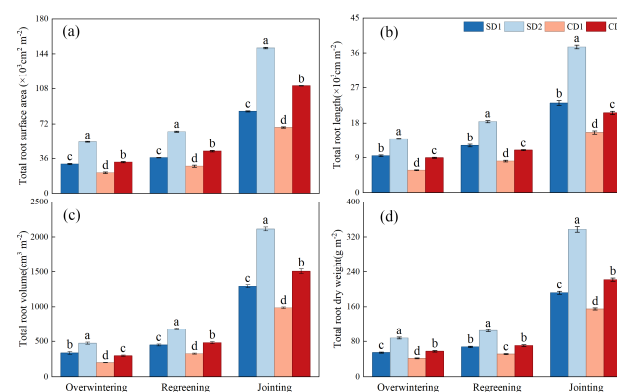


Figure 8. Effects of soil type and basic seedling density on root morphology per unit area in wheat in 2024–2025. Note: (a) total root surface area ($\text{cm}^2 \text{ m}^{-2}$); (b) total root length (cm m^{-2}); (c) total root volume ($\text{cm}^3 \text{ m}^{-2}$); (d) total root dry weight (g m^{-2}). SD1, sandy soil + low basic seedling density (2.8×10^6 grains hm^{-2}); SD2, sandy soil + high basic seedling density (4.5×10^6 grains hm^{-2}); CD1, clay soil + low basic seedling density (2.8×10^6 grains hm^{-2}); CD2, clay soil + high basic seedling density (4.5×10^6 grains hm^{-2}). Different lowercase letters indicate significant differences among treatments at the same growth stage at $p < 0.05$. Error bars represent standard deviation ($n = 3$).

Under the same basic seedling density, root morphology indices per unit area of wheat increased significantly when soil type changed from clay to sandy soil. Compared with clay soil, sandy soil under the identical basic seedling density significantly improved the two-year average root morphology indicators at overwintering, regreening, and jointing stages: total root length increased by 57.54%, 63.50%, and 68.02%; total root surface area rose by 53.42%, 41.02%, and 31.42%; total root volume grew by 64.08%, 39.85%, and 37.05%; and total root dry weight was enhanced by 43.48%, 41.92%, and 42.42%, respectively.

3.10. Responses of Wheat Root Activity to Soil Type and Basic Seedling Density

Soil type and basic seedling density exerted extremely significant effects on wheat root activity across all growth stages, whereas significant interaction between soil type and basic seedling density were detected at the regreening and jointing stages (Table 10). Under identical soil type, root activity of wheat increased significantly with the increase in basic seedling density. Compared with D1, D2 under identical soil type improved root activity at all growth stages across the two experimental years, with a more prominent increment observed under sandy soil conditions, while the increases were relatively moderate at regreening and jointing stages under clay soil conditions. On the two-year average basis, compared with D1, root activity of D2 under sandy soil conditions increased by 9.51%, 8.71%, and 11.50% at overwintering, regreening, and jointing stages, respectively. Under clay soil conditions, the corresponding increments of D2 were 7.40%, 2.88%, and 4.35%. Under the same basic seedling density, root activity of wheat differed significantly with varying soil type at the regreening and jointing stages. Compared with clay soil, sandy soil with the same basic seedling density significantly increased wheat root activity at the regreening and jointing stages over the two experimental years. Under D1 treatment, the two-year average root activity under sandy soil conditions was increased by 6.43% and 6.20% at regreening and jointing stages relative to clay soil conditions; the corresponding increments under D2 reached 12.45% and 16.28%, respectively.

Table 10. Effects of soil type and basic seedling density on root activity in wheat.

Years	Treatment	Overwintering ($\mu\text{g g}^{-1}$ FW h^{-1})	Regreening ($\mu\text{g g}^{-1}$ FW h^{-1})	Jointing ($\mu\text{g g}^{-1}$ FW h^{-1})
2024	SD1	58.30 b	108.27 b	135.82 b
	SD2	62.93 a	119.13 a	154.94 a
	CD1	50.79 d	102.13 c	130.47 c
	CD2	54.86 c	105.91 bc	133.13 bc
2025	SD1	55.87 b	109.19 b	137.29 b
	SD2	62.10 a	117.26 a	153.29 a
	CD1	50.59 c	102.19 c	126.70 c
	CD2	54.02 b	104.30 bc	131.94 bc
Year (Y)		ns	ns	ns
Soil (S)		196.70 **	74.34 **	154.74 **
Density (D)		79.22 **	29.62 **	81.97 **
Y $\times$ S		ns	ns	ns
Y $\times$ D		ns	ns	ns
S $\times$ D		ns	8.18 *	32.82 **
Y $\times$ S $\times$ D		ns	ns	ns

Note: SD1, sandy soil + low basic seedling density (2.8×10^6 grains hm^{-2}); SD2, sandy soil + high basic seedling density (4.5×10^6 grains hm^{-2}); CD1, clay soil + low basic seedling density (2.8×10^6 grains hm^{-2}); CD2, clay soil + high basic seedling density (4.5×10^6 grains hm^{-2}). Different letters indicated significant difference between treatments at the 0.05 probability level. * and ** indicate significant differences among levels within a factor at the 0.05 and 0.01 levels, respectively; ns indicates no significant difference.

3.11. Responses of Nitrogen Utilization Enzymes in Wheat Roots to Soil Type and Basic Seedling Density

Soil type and basic seedling density both significantly affected the activities of nitrogen utilization enzymes in wheat (Table 11). Soil type and basic seedling density showed signif-

icant interactive effects on NiR and GOGAT activities at both the heading and mid-grain filling stages, indicating that the combined influence of soil and density was consistently important for these two enzymes across the two periods. By contrast, GS activity was affected by this interaction only at the mid-grain filling stage, with no such interactive effect observed at the heading stage. Under clay soil conditions, the activities of NR and NiR in wheat declined with the increase in basic seedling density. Compared with D1, the two-year average activities of NR and NiR in D2 decreased by 16.54% and 26.12% at heading stage, with respective reductions of 18.84% and 12.78% at mid-filling stage. Under sandy soil conditions, the activities of NR and NiR also declined with increased basic seedling density. Relative to D1, the two-year average NR and NiR activities of D2 decreased by 28.74% and 13.30% at the heading stage, and fell by 29.55% and 22.73% at the mid-filling stage, respectively.

Table 11. Effects of soil type and basic seedling density on nitrogen utilization enzymes in wheat roots.

Years	Treatment	Heading ($\mu\text{mol g}^{-1} \text{FW h}^{-1}$)				Mid-Grain Filling ($\mu\text{mol g}^{-1} \text{FW h}^{-1}$)			
		NR	NiR	GS	GOGAT	NR	NiR	GS	GOGAT
2024	SD1	0.47 c	2.92 c	5.19 c	3.59 b	0.23 c	0.93 c	4.77 c	3.05 c
	SD2	0.35 d	2.63 d	4.53 d	2.79 c	0.17 d	0.72 d	3.72 d	2.50 d
	CD1	0.66 a	4.50 a	6.73 a	4.02 a	0.36 a	1.18 a	6.38 a	3.76 a
	CD2	0.59 b	3.17 b	6.30 b	3.73 b	0.28 b	1.01 b	5.62 b	3.42 b
2025	SD1	0.40 c	2.72 b	4.93 c	3.44 c	0.21 c	0.83 c	4.68 c	2.91 c
	SD2	0.27 d	2.26 d	4.44 d	2.64 d	0.14 d	0.64 d	3.65 d	2.31 d
	CD1	0.67 a	3.12 a	6.53 a	3.72 a	0.33 a	1.09 a	6.16 a	3.63 a
	CD2	0.52 b	2.46 c	5.83 b	3.57 b	0.28 b	0.97 b	5.27 b	3.12 b
Year (Y)		90.03 **	431.25 **	14.73 **	50.31 **	42.02 **	60.00 **	22.88 **	34.49 **
Soil (S)		1819.56 **	451.18 **	563.98 **	583.17 **	1640.88 **	806.72 **	1878.35 **	600.11 **
Density (D)		412.70 **	463.64 **	74.01 **	362.89 **	387.52 **	286.39 **	603.13 **	237.11 **
Y $\times$ S		22.08 **	141.17 **	ns	ns	ns	ns	7.40 *	ns
Y $\times$ D		17.26 **	14.58 **	ns	ns	ns	ns	ns	ns
S $\times$ D		ns	92.87 **	ns	118.14 **	ns	8.08 *	7.96 *	5.77 *
Y $\times$ S $\times$ D		9.88 **	42.65 **	ns	ns	10.35 **	ns	ns	ns

Notes: SD1, sandy soil + low basic seedling density (2.8×10^6 grains hm^{-2}); SD2, sandy soil + high basic seedling density (4.5×10^6 grains hm^{-2}); CD1, clay soil + low basic seedling density (2.8×10^6 grains hm^{-2}); CD2, clay soil + high basic seedling density (4.5×10^6 grains hm^{-2}). NR, nitrate reductase; NiR, nitrite reductase; GS, glutamine synthetase; GOGAT, glutamate synthase. Different letters indicated significant difference between treatments at the 0.05 probability level. * and ** indicate significant differences among levels within a factor at the 0.05 and 0.01 levels, respectively; ns indicates no significant difference.

Under D1 treatment, the activities of four nitrogen utilization enzymes decreased significantly at both stages when soil type changed from clay to sandy soil. The two-year average activities of NR, NiR, GS, and GOGAT under SD1 were reduced by 34.59%, 25.98%, 23.68%, and 9.17%, respectively compared with CD1 at the heading stage. Significant soil type effects persisted at the mid-filling stage, with SD1 showing respective declines of 36.23%, 22.47%, 24.64%, and 19.35% relative to CD1; notably, the reduction range of GOGAT increased markedly during mid-filling. Under D2 treatment, the inhibitory effect on nitrogen utilization enzyme activities was more pronounced when soil type shifted from clay to sandy soil. At the heading stage, the activities of NR, NiR, GS, and GOGAT in SD2 were decreased by 44.14%, 13.14%, 26.05%, and 25.62%, respectively, compared with CD2. At the mid-filling stage, except for NiR, the reduction amplitudes of the other three enzymes remained at high levels or further increased: NR, GS, and GOGAT declined by 44.64%, 32.32%, and 26.45%, while NiR decreased by 31.31%.

4. Discussion

4.1. Effects of Soil Type and Basic Seedling Density on Wheat Yield and Its Components

Under late-sown conditions, reduced accumulated temperature before winter restrains tillering and decreases total tiller population, leading to insufficient spike-bearing tillers [27,28]. In this study, SD2 (4.5×10^6 grains hm^{-2}) achieved the maximum grain yield. Within sandy soil, grain yield under D2 was significantly increased by 5.65% compared with D1; within clay soil, the increase was 2.84% and statistically non-significant; however, the effects of the soil type $\times$ density interaction were not detected. The yield advantage of D2 in sandy soil was mainly attributed to the substantially elevated spike population, which facilitated the formation of spike-bearing tillers (Table 4). Clay soil features poor air permeability and high mechanical impedance, which impedes root penetration. Meanwhile, late sowing shortens the effective growing period and restricts the growth duration of both root and aboveground organs [29]. Significant $S \times D$ interactions were detected for tiller number at jointing, booting, and maturity stages, as well as for spike-bearing tiller percentage (Table 5), confirming that soil type influenced the density response for tiller dynamics. Under sandy soil conditions, wheat tillering capacity was improved with significantly higher tiller population. Compared with clay soil, tiller number increased by 5.69% and 6.53% at jointing stage, respectively. Meanwhile, sandy soil markedly promoted the spike-bearing tiller percentage and secured sufficient spike-bearing tillers (Table 5). At the same basic seedling density, grain number per spike was higher under sandy soil than under clay soil (Table 4). The $S \times D$ interaction for grains per spike was significant (Table 4), indicating that the positive density effect on this component was stronger in sandy soil than in clay soil. The sandy soil mitigates the adverse effects of high density through improved aeration, rooting space, and thermal conditions, enabling the density-induced population advantage to be better expressed in terms of spike grain number, whereas the physical constraints of clay soil become increasingly limiting as density rises, weakening the density effect.

4.2. Effects of Soil Type and Basic Seedling Density on Photosynthetic Characteristics of Wheat

Elevated basic seedling density significantly increased wheat LAI and LAD (Table 7), with SD2 maintaining higher post-anthesis dry matter accumulation. The $S \times D$ interaction was not significant for LAD or aboveground dry matter accumulation (Tables 6 and 9), indicating that the density-induced increase in canopy photosynthetic area was a consistent response across both soils. The improvement in canopy dry matter accumulation induced by increased basic seedling density is usually accompanied by reduced individual leaf photosynthetic efficiency, and these two indicators cannot be optimized simultaneously [30]. In this study, despite greater canopy dry matter accumulation under SD2, the single-leaf P_n , G_s , and rubisco activity were all significantly lower relative to SD1 (Figure 3). The increase in basic seedling density from D1 to D2 intensified interplant competition for light and nitrogen, inhibiting rubisco synthesis. Because the $S \times D$ interaction was not significant for canopy photosynthetic rate (Table 9) or dry matter accumulation (Table 6), the compensatory effect of increased leaf area occurred in both soils, and the higher absolute values under sandy soil could not reflect the main effect of soil type. Although CD2 also increased LAI and LAD under clay soil conditions, its canopy photosynthetic rate was significantly lower than that of CD1 (Table 9). However, because the $S \times D$ interaction was not significant for canopy photosynthetic rate or the proportion of post-anthesis dry matter accumulation, this reduction reflects a main effect of soil type rather than a soil-dependent exacerbation of the density effect. Compared with sandy soil, wheat grown in clay soil exhibited significantly lower P_n , G_s , and rubisco activity at both heading and mid-filling stages, which may be related to restricted root activity under late-sown conditions in clay

soil. SD2 exhibited elevated SPAD but reduced rubisco activity relative to SD1 and CD1 (Table 8), reflecting an adaptive trade-off under resource limitation: prioritizing light-harvesting over carbon assimilation [31]. This strategy was particularly prominent under sandy soil, where enlarged leaf area sustained canopy-scale carbon assimilation despite reduced rubisco activity.

4.3. Effects of Soil Type and Basic Seedling Density on Root Morphology and Physiological Characteristics of Wheat

Clay soil generally possesses stronger nutrient retention capacity [32]. In this study, the initial concentrations of total nitrogen, organic matter, and available potassium in clay soil were higher than those in sandy loam across both the 2023–2024 and 2024–2025 growing seasons, supplying relatively sufficient nutrient reserves for wheat growth. Nevertheless, total root biomass under clay soil was markedly lower than that in sandy loam. This can be explained by the greater mechanical impedance of clay soil, which impedes root penetration and elongation. Consequently, the ample inherent soil nutrients become less available for wheat absorption and utilization, offsetting the potential growth advantage derived from the high background fertility of clay soil [14]. In contrast, sandy loam has a well-developed pore structure and favorable aeration, which effectively relieve mechanical limitations to root growth and promote root elongation and branching [32,33]. Under late-sowing conditions, the shortened growth period and inadequate accumulated temperature suppress root development. In addition, elevated basic seedling density intensifies competition for belowground resources. The cooccurrence of late sowing and high density imposes stronger constraints on individual root development [34,35]. In the present study, compared with the low-density treatment, the high-density treatment decreased individual plant root dry weight by 15.05% and 15.66%, total root surface area by 4.63% and 11.85%, and total root volume by 14.82% and 9.65% in the two experimental years, respectively. Owing to the favorable physical structure of sandy loam, individual root growth remained superior in sandy loam relative to clay soil even when planting density increased. Nevertheless, total root length, root surface area, root volume, and root dry weight per unit area increased significantly with the rise in basic seedling density, indicating compensatory accumulation of total root biomass at population level. Such compensatory effect was more pronounced in sandy soil, further demonstrating that sandy soil enables roots to sustain greater total absorbing area under density stress. Root activity serves as a key physiological indicator for evaluating root metabolic capacity [36]. Under sandy soil conditions, increasing basic seedling density significantly improved wheat root activity; compared with clay soil, root activity was markedly elevated by 8.80–16.35% (Table 10). Under clay soil conditions, increasing density failed to improve root activity of late-sown wheat. Clay soil sustained higher nitrogen utilization enzyme activities (NR, NiR, GS, GOGAT) than sandy soil (Table 11), attributed to its superior water and nitrogen retention capacity. However, this nitrogen metabolic advantage could not offset the disadvantages of constrained photosynthetic productivity and root development under clay soil conditions.

5. Conclusions

Increasing basic seedling density enhanced grain yield, overall, with the increase being statistically significant only under sandy soil conditions; it optimized population structure, boosting tiller dynamics and spike-bearing tiller percentage, and elevated leaf area index. Under clay soil, however, these positive effects were weak or absent. Despite higher nitrogen utilization enzyme activities (NR, NiR, GS, GOGAT) from heading to mid-filling in clay soil, this advantage could not compensate for insufficient photosynthetic productivity and restricted root development, failing to translate into yield benefits. Notably, elevated

density expanded canopy photosynthetic area but suppressed single-leaf photosynthetic capacity, with this individual-population divergence being particularly pronounced under high-density clay soil treatment. In conclusion, the yield response of late-sown wheat to increased basic seedling density is strongly modulated by soil type, with photosynthetic characteristics and root physiology showing greater sensitivity to density variation under sandy soil. Additionally, although the soil type $\times$ density interaction was not significant for grain yield, the numerical yield increase was greater in sandy soil, and significant interactions were observed for grains per spike, effective spikes, tiller number (except at overwintering), and LAI at maturity. These findings, derived from a single cultivar (Yangmai 25), single late sowing date per season, and plot-scale cement bins, require multi-site, multi-year validation under field-scale conditions before broad agronomic application.

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