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Synergistic Optimization of Root–Shoot Characteristics, Nitrogen Use Efficiency and Yield by Combining Planting Density with Nitrogen Level in Cotton (*Gossypium hirsutum* L.)

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Abstract

To address low nitrogen use efficiency (NUE) derived from excessive fertilization in cotton production in the Yellow River Basin, a field study was conducted to evaluate the effects of two planting densities and six nitrogen (N) rate levels. Key results show that a N rate of 225 kg ha^{-1} optimized root length density and root biomass density. High planting density (105,000 plants ha^{-1}) improved the population-level root traits, photosynthetic radiation interception, and boll number per unit area, though it reduced individual plant root development. Total dry matter peaked at 225 kg ha^{-1} N, and density increased reproductive dry matter by 7.5–11.9%. Higher N rates reduced reproductive partitioning and root–shoot ratio. While the maximum seed cotton yield (SCY) was 225 kg ha^{-1}, near-maximum yield was achieved at 150 kg ha^{-1}. NUE declined with increasing N, but densification improved agronomic NUE and partial factor productivity by 1.5–6.6% and 3.3–39.3%, respectively. Under the "densification with N reduction" mode, combining a planting density of 105,000 plants·ha^{-1} with an N rate of 150 kg·ha^{-1} achieved conventional yield. At the same density, an N rate of 225 kg·ha^{-1} not only enabled high yield and maintained relatively high NUE but also showed better adaptability to the simplified cultivation mode in Yellow River Basin cotton-growing regions.

Keywords: planting density; nitrogen application rate; root–shoot characteristics; nitrogen fertilizer utilization efficiency; seed cotton yield

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1. Introduction

Cotton is an important economic crop in China and China also holds a key position in the global cotton industry [1]. However, in China, in pursuit of high cotton yields, excessive fertilization occurs, far exceeding the actual demand of crops, and the utilization rate of nitrogen fertilizer is significantly lower than that in developed countries [2,3]. Optimizing planting density and improving nitrogen fertilizer utilization efficiency have become two of the key measures to increase cotton yield [4,5].

Nitrogen plays a key role in the growth and development of plants and is an important factor for ensuring crop growth and increasing yield and quality [6,7]. Appropriate nitrogen fertilizer application can effectively regulate the source–reservoir relationship of crops and optimize the accumulation and distribution of dry matter [8]. However, improper application of nitrogen fertilizer can have adverse effects on crop growth: excessive nitrogen application can easily lead to cotton plants being overly green and late-maturing [9], reducing the proportion of reproductive organs and thus lowering the yield; insufficient nitrogen fertilizer accelerates leaf senescence, significantly inhibits the accumulation of dry matter in cotton plants and thereby affects cotton yield and quality [10]. Crops can enhance nitrogen utilization efficiency by optimizing their root system structure. For example, corn can enhance nitrogen absorption by expanding the contact range between the root system and the soil [11]. Appropriate nitrogen application can promote root development [12,13]. However, for cotton, excessively high nitrogen levels can inhibit root growth and lead to nitrogen leaching loss [14–16], reducing nitrogen fertilizer utilization efficiency. In contrast, low nitrogen stress will limit root elongation and reduce the average root diameter, thereby reducing the nitrogen absorption capacity of cotton [17]. In conclusion, both insufficient and excessive nitrogen supply will have adverse effects on crop yield [18,19]. Therefore, reasonable regulation of nitrogen fertilizer application is of great significance for maintaining high and stable crop yields.

Increasing planting density is an important way to achieve high yields of field crops [20]. Under low-nitrogen conditions, increasing density can significantly increase SCY in field-cultivation conditions [21]. Appropriate densification leads to a decrease in the number and weight of bolls per plant, but the population SCY significantly increases [22,23]. Although a higher nitrogen application rate can promote leaf area per plant and photosynthetic capacity [24], Chen et al. pointed out that increasing density is more conducive to improving the total biomass of crop populations [25]. Similar phenomena have also been observed in corn plants: under traditional fertilization conditions, moderately increasing density can improve the efficiency of light energy and nutrient utilization, and enhance leaf photosynthesis and dry matter accumulation, thereby increasing grain yield at population level [26]. Nutrient absorption by the root system directly determines the formation of biomass and yield. For example, studies on rapeseed have found that after increasing density, the root length, root surface area and root volume of individual plants decreased, but the distribution of the population root system was improved [27,28]. In conclusion, optimizing planting density not only enhances the photosynthetic capacity of crop populations but also improves nutrient absorption efficiency, thereby maximizing the yield potential of the population [29].

With the development of the simplified cultivation model in the cotton-growing areas of the Yellow River Basin, the cotton planting density has gradually increased from 60,000 plants per hectare in the early stage to 90,000 plants per hectare [30,31]. To adapt to the highly efficient full-mechanization production mode, there is still some room for improving the planting density.

To address the prevalent issue in China's cotton production, where excessive fertilization, driven by the pursuit of high yields, results in low NUE, this study explores a technical approach to enhancing NUE by increasing planting density while reducing N fertilizer application. The objectives of this study are as follows: (1) to identify the optimal N application rate under the density-increased cultivation pattern; (2) to decipher the mechanisms underlying cotton growth and development regulated by this pattern; (3) to clarify the mechanisms through which this pattern improves cotton yield.

2. Materials and Methods

2.1. Experimental Design

This study was conducted from 2022 to 2023 at the Weixian Experimental Station (115.35° E, 37.08° N), College of Agriculture, Hebei Agricultural University, Hebei Province, China—on the basis of an 8-year (2014–2021), long-term N-positioning experimental field. The site is located in a temperate monsoon climate zone. Meteorological data during the cotton-growing season (April–October) are presented in Figure 1: in 2022, the average daily temperature was 15.4 °C and total rainfall was 700.6 mm; in 2023, the corresponding values were 14.8 °C and 677.9 mm, respectively.

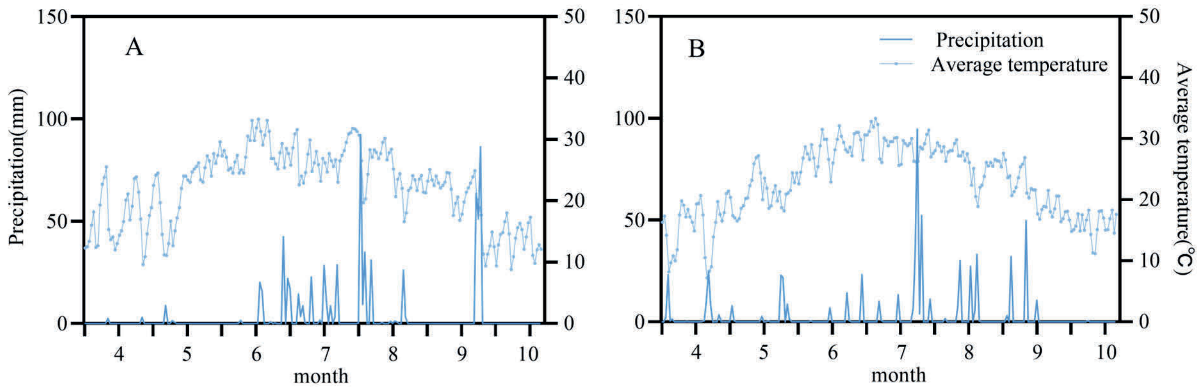

Figure 1. Daily average temperature and precipitation in 2022 (**A**) and 2023 (**B**).

Upland cotton (*Gossypium hirsutum* L.) variety "nongda 36" was used as the experimental material. A split-plot design was employed, with planting density as the main plot factor and N application rate as the sub-plot factor (Table 1). For the main plot (planting density), two levels were set up: low density (D6: 60,000 plants ha^{-1}) and high density (D10.5: 105,000 plants ha^{-1}). For the sub-plot (N application rate), six rates were established: 0 kg N ha^{-1} (N0), 75 kg N ha^{-1} (N1), 150 kg N ha^{-1} (N2), 225 kg N ha^{-1} (N3), 300 kg N ha^{-1} (N4) and 375 kg N ha^{-1} (N5).

Table 1. Experimental treatment.

Treatment	N (kg N ha^{-1})	Planting Density (Plants ha^{-1})
D6N0	0	60,000
D6N1	75	60,000
D6N2	150	60,000
D6N3	225	60,000
D6N4	300	60,000
D6N5	375	60,000
D10.5N0	0	105,000
D10.5N1	75	105,000
D10.5N2	150	105,000
D10.5N3	225	105,000
D10.5N4	300	105,000
D10.5N5	375	105,000

A randomized complete block design was adopted for the experiment, with three replications per treatment and each plot having an area of 66 m^2. Urea (46% N) was used as the N source, applied as basal fertilizer (60%) and topdressing (40%), which was conducted on 8 July in both experimental years (2022–2023). Sowing was performed on 25 April (both years) with a uniform row spacing of 76 cm. Basic fertility properties of the experimental soil (0–60 cm depth) are presented in Table 2. All other agronomic practices were conducted to be consistent with the local high-yield cotton production standards.

Table 2. Soil physicochemical indicators.

Treatment	TN (g/kg)	AHN (mg/kg)	AP (mg/kg)	AK (mg/kg)	SOM (g/kg)
N0	0.53	42.55	9.14	225.00	8.21
N1	0.58	51.28	9.18	230.00	8.46
N2	0.57	54.28	9.24	236.67	8.79
N3	0.62	61.39	9.36	241.39	9.50
N4	0.63	61.54	9.43	243.89	9.57
N5	0.63	61.64	9.71	245.28	9.60

TN: Total N, AHN: Alkaline Hydrolyzable N, AP: Available Phosphorus, AK: Available Potassium, SOM: Soil Organic Matter.

2.2. Aboveground Part Indicators

Plant height (PH): During the flowering and bolling stage (80 DAS) and the boll opening stage (110 DAS), select 6 cotton plants with uniform growth and use a ruler to measure the length from the cotyledon node to the growth point of the main stem.

Stem diameter (SD): During the flowering and bolling stage (80 DAS) and the boll opening stage (110 DAS), select 6 cotton plants with uniform growth. Measure the diameter of the main stem 1 cm above the cotyledon node using a vernier caliper.

The photosynthetically active radiation (PAR) at different parts of the canopy of the marked plants was measured during the bud formation stage, the initial flowering stage, the full flowering stage and the boll opening stage. This was performed on a clear and cloudless day and the measurements were conducted from 10:00 to 14:00. The PAR transmission characteristics of the canopy were determined using a canopy analyzer (SUNSCAN, Delta, UK). The probe was placed 30 cm above the top of the canopy, with vertical upward measurement for incident PAR at the top of the canopy (PARI) and vertical downward measurement for reflected PAR from the canopy (PARR). Then, the probe was placed at the bottom of the canopy to measure the incident PAR at the bottom (PART) with vertical upward orientation. The PAR interception rate (PARIn) is calculated using the following formula:

$$\text{PARIn} = (\text{PARI} - \text{PART} - \text{PARR})/\text{PARI}$$

2.3. Root System Characteristics

The root structure images of cotton were collected during the boll opening stage (110 DAS) [32]. Three representative cotton plants were selected from each plot using a standard shovel. A soil block of 20 cm × 55 cm × 40 cm (plant spacing × row spacing × depth) was dug out around the root system. The freshly dug root system was gently shaken to remove large soil particles adhering to it. Then, a low-pressure water gun was used to wash away residual soil particles on the root system. The root system was imaged using an image acquisition device (Daheng Imaging, GigE Vision TL, Beijing, China). The obtained root system images were analyzed with Root Nav 2.0 software to determine the average lateral root angle (ALRA) and the number of primary lateral roots (NLR).

At the flowering and bolling stage (80 DAS) and the boll opening stage (110 DAS), three cotton plants with uniform growth were selected, and samples were taken between rows 5 cm away from the cotton plants. A hard soil root drill with an inner diameter of 70 mm was used to take soil blocks containing roots, divided into four layers (0–15, 15–30, 30–45 and 45–60 cm). The soil clods containing roots were washed to obtain the root systems, which were then placed on a root plate (44 cm × 31 cm × 3 cm) containing

0.5 cm of water, ensuring no overlap between root systems. The root systems were then scanned (300 dpi) using a scanner (EPSON Expression 10000XL; Seiko Epson Corporation., Suwa, Nagano, Japan) to obtain root system images. After scanning, the root systems were dried at 85 °C to constant weight and weighed to determine root biomass. The scanned root system images were analyzed using WinRHIZO REG 2009 (Regent Instruments, Inc., Quebec City, QC, Canada) to obtain total root length (TRL). Root length density (RLD) and root biomass density (RBD) were calculated using Formulas (1) and (2):

$$\text{Root length density } (\text{cm}\cdot\text{cm}^{-3}) = \text{root length}/\text{soil volume} \tag{1}$$

$$\text{Root biomass density } (\text{mg}\cdot\text{cm}^{-3}) = \text{root biomass}/\text{soil volume} \tag{2}$$

2.4. Dry Matter Accumulation of Cotton Plants

Three representative cotton plants were harvested per plot for each treatment to determine dry matter accumulation. Harvested plants were separated into reproductive organs and vegetative organs. All plant parts were first deactivated in a forced-air oven at 105 °C for 30 min, then subsequently dried at 80 °C to a constant weight. After cooling to room temperature, each organ fraction was weighed using an electronic balance.

2.5. Yield and Its Components

Cotton yield and its components: Two rows in the middle of each plot were selected, with 20 representative plants continuously chosen in each row for harvesting. The number of harvested bolls was recorded to determine the number of bolls per plant and single boll weight. The seed cotton yield (SCY) was obtained by weighing, and the lint percentage was calculated after ginning.

2.6. Nitrogen Fertilizer Utilization Efficiency

Nitrogen Recovery Efficiency (NRE): The average value of N uptake across three replicates in the non-N-applied plots was used as the N uptake of the non-N-applied plots in the formula.

Nitrogen Recovery Efficiency (NRE) = (Nitrogen Uptake in Nitrogen-Fertilized Plot − Nitrogen Uptake in Nitrogen-Unfertilized Plot)/Nitrogen Application Rate

Agricultural nitrogen use efficiency (ANUE): The average SCY across three replicates of the non-N-applied plots was used as SCY of the non-N-applied plots in the formula.

Agricultural nitrogen use efficiency (ANUE) = (SCY in nitrogen-applied plots − SCY in non-nitrogen-applied plots)/Nitrogen application rate

Partial factor productivity of nitrogen (PFPN) = SCY in nitrogen-applied plots/Nitrogen application rate

2.7. Total Soil Nitrogen Content at Different Growth Stages

Soil total nitrogen (STN) was determined at two cotton growth stages: the flowering and bolling stage (80 DAS) and the boll opening stage (110 DAS). A five-point diagonal sampling method was used, with one soil core collected at each of five points per plot using a stainless-steel soil auger. Each soil core was divided into three depth layers: 0–20 cm, 20–40 cm and 40–60 cm. Soil samples from each layer were first air-dried naturally in a well-ventilated, dust-free room. After removing visible impurities, samples were ground and passed through a 60-mesh (0.25 mm) sieve. Approximately 0.5 g of sieved soil was digested via the H_2SO_4-H_2O_2 digestion method. The STN content of the digested solution was then measured using a continuous flow analyzer (Model: AutoAnalyzer 3; Manufacturer: SEAL Analytical, Mequon, WI, USA).

2.8. Data Analysis

Data recording and preliminary organization were performed in Microsoft Excel 2013 (Microsoft Corporation, Redmond, WA, USA). Prior to data processing, normality tests, including the Kolmogorov–Smirnov test and the Shapiro–Wilk test, were performed. Statistical analysis of experimental data was conducted via one-way analysis of variance (ANOVA) using SPSS Statistics 27.0 (IBM Corporation, Armonk, NY, USA). Differences in mean values among treatment groups were compared using the least significant difference (LSD) test at a significance level of $p < 0.05$ (5%). Correlation analysis was performed in Origin 2024 (OriginLab Corporation, Northampton, MA, USA). Experimental results were visualized as graphs using GraphPad Prism 8.4.0 (GraphPad Software, Inc., San Diego, CA, USA) and Adobe Illustrator 2021 (Adobe Software, Inc., San Jose, CA, USA). Partial least squares path modeling (PLS–PM) was performed in R (version 4.4.3). Through the seed cotton yield (y) corresponding to each nitrogen application rate (x), the regression equation was obtained using the quadratic polynomial regression model in Microsoft Excel 2013.

3. Results

3.1. The Influence on the Aboveground Morphology of Cotton

In the first year of the experiment, with the increase in N application rate, PH showed a gradual upward trend at the flowering and bolling stage. At the boll opening stage, PH reached the maximum value under the N3 treatment, with no significant difference from that under the N5 treatment (Figure 2). For the same N application rate, at the flowering and bolling stage, PH under the D10.5 planting density was significantly higher than that under the D6 density ($p < 0.05$), with increases of 16.2% (N0), 13.5% (N1), 11.2% (N2), 10.4% (N3), 9.1% (N4) and 7.3% (N5), respectively. At the boll opening stage, the difference in PH between the two planting densities narrowed, with only the difference under the N0 treatment being significant ($p < 0.05$), while no significant differences were observed under the N1–N5 treatments. The above trends were generally consistent across the two experimental years. Furthermore, both planting density and N application rate had significant effects on cotton PH.

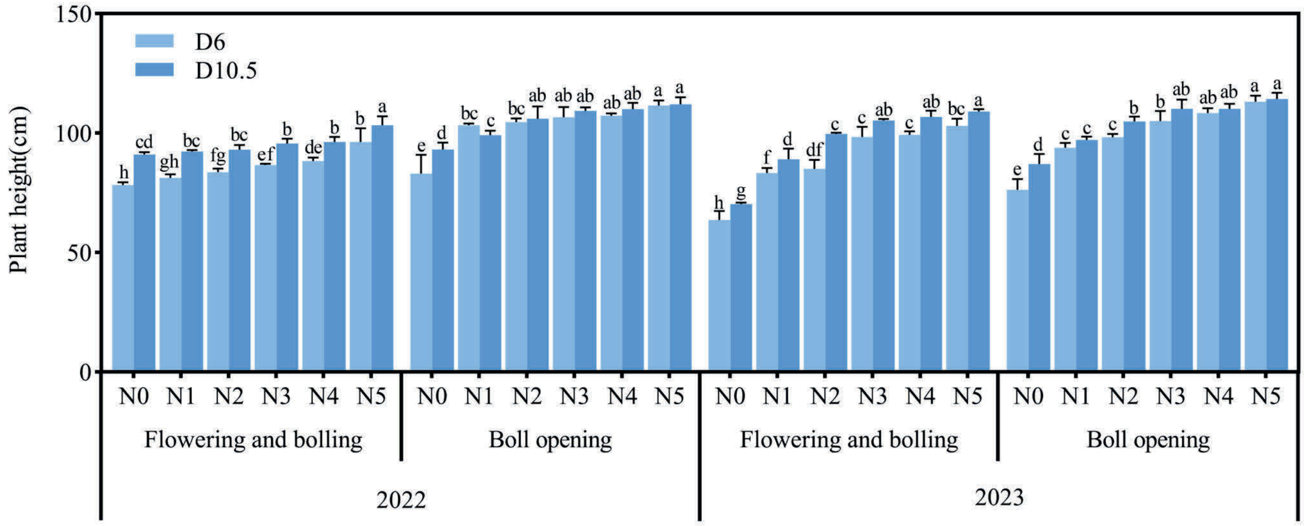

Figure 2. Cotton PH under different planting densities and N application rates (2022–2023). Different letters indicate significant differences among treatments at $p < 0.05$. Error bars represent the standard error (SE).

During the first experimental year (Figure 3), under the D10.5 density, no significant differences in SD were observed among N treatments. Under the D6 density, N application significantly increased SD ($p < 0.05$), with the smallest value in N0. At the flowering and bolling stage, compared with N0, SD in N1–N5 increased by 21.3% (N1), 31.6% (N2), 33.1% (N3), 40.8% (N4) and 50.7% (N5), respectively. At the boll opening stage (110 days after sowing), the increases were 30.4% (N1), 32.2% (N2), 31.6% (N3), 31.3% (N4) and 41.7% (N5) for N1–N5, respectively. For the same N rate, SD under D6 was significantly higher than that under D10.5 ($p < 0.05$). At the boll opening stage, the increases in D6 relative to D10.5 were 17.6% (N1), 19.0% (N2), 16.1% (N3), 10.7% (N4) and 18.8% (N5), respectively. In conclusion, increasing density reduced cotton SD, while increasing N application increased it. Both factors had significant effects on cotton SD.

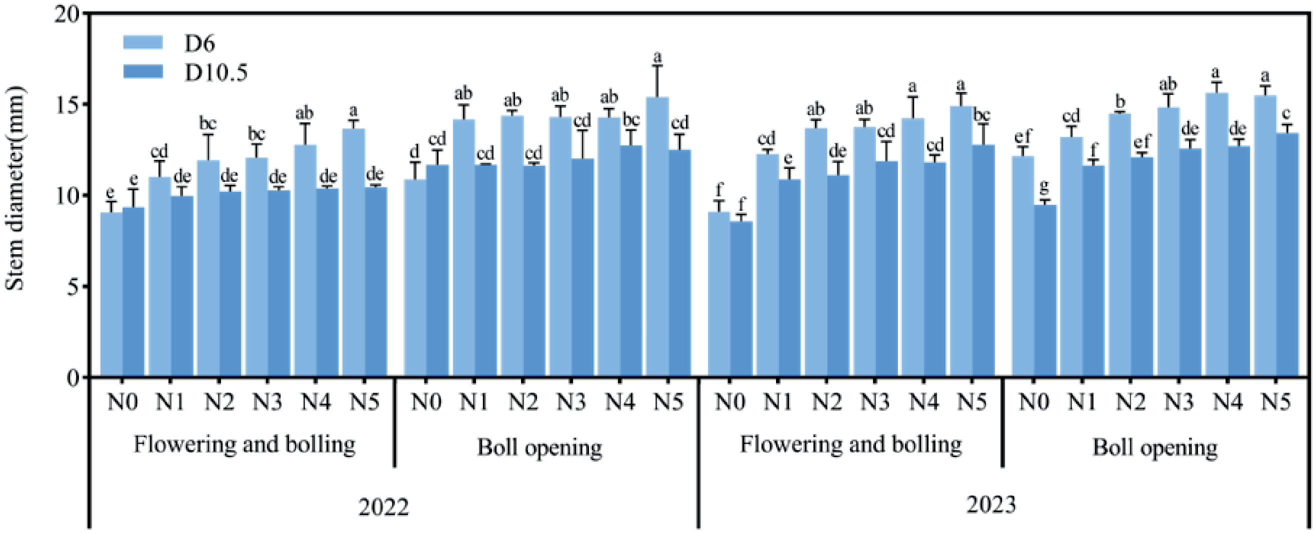

Figure 3. Cotton SD under different planting densities and N application rates (2022–2023). Different letters indicate significant differences among treatments at $p < 0.05$. Error bars represent the standard error (SE).

During the cotton squaring stage, both increasing N application rate and planting density significantly improved the population PARIn ($p < 0.05$) (Figure 4A). Under D10.5, compared to the high-density N0 treatment, the PARIn of the N1–N5 treatments increased by 38.9% (N1), 30.5% (N2), 20.6% (N3), 25.8% (N4) and 26.0% (N5), respectively—with the greatest relative increase observed in the N3 treatment. As the growth period progressed, the differences in PARIn among different treatments gradually narrowed during the early flowering stage (Figure 4B), full flowering stage (Figure 4C), and boll opening stage (Figure 4D).

3.2. Effects on Root System Characteristics

In the first year of the experiment, with increasing N application rate, the ALRA of cotton first increased and then stabilized. At the same N application rate, the ALRA under the D10.5 treatment was lower than that under D6, but the difference between the two densities was not significant ($p > 0.05$) (Figure 5A). Figure 5B illustrates that the NLR also exhibited a trend of initial increase followed by stabilization with increasing N application rate. Among all treatments, the NLR in the D6N0 and D10.5N0 treatments was significantly lower than that in other N-applied treatments ($p < 0.05$), and the maximum number was observed in the D6N3 and D10.5N3 treatments. In addition, at the same N application rate, the NLR under D6 was slightly higher than that under D10.5, but the difference was not significant ($p > 0.05$). Statistical analysis revealed that planting density, N application rate, and their interaction had no significant effect on the ALRA of cotton, but exerted a significant effect on the NLR.

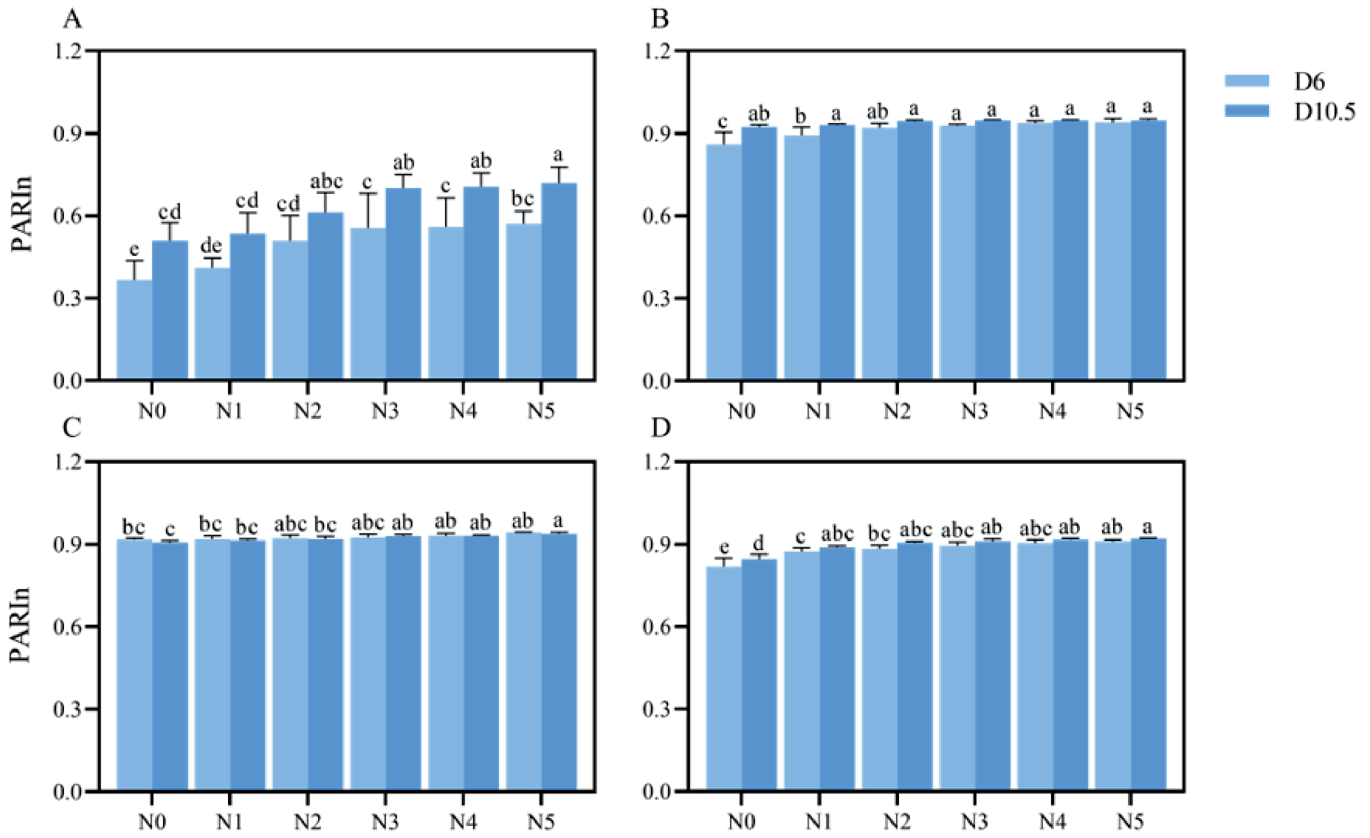

Figure 4. PARIn in cotton fields under different planting densities and N application rates (2023). Squaring stage (**A**), early flowering stage (**B**), full flowering stage (**C**), and boll opening stage (**D**). Different letters indicate significant differences among treatments at $p < 0.05$. Error bars represent the standard error (SE).

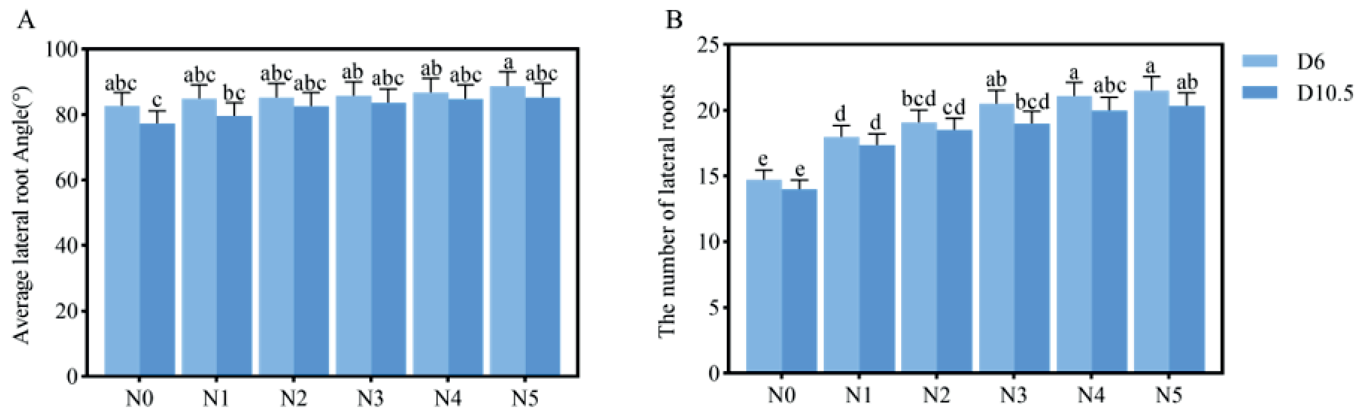

Figure 5. Effects of different planting densities and N application rates on ALRA (**A**) and NLR (**B**) of cotton root systems (2022). Different letters indicate significant differences among treatments at $p < 0.05$. Error bars represent the standard error (SE).

During the first experimental year, cotton RLD decreased with increasing soil depth, with 80.8–86.5% of the total root length concentrated in the 0–45 cm soil layer (Figures 6 and 7). Under the same planting density, per-plant RLD exhibited a unimodal curve (first increasing and then decreasing) with increasing N application rate, reaching the maximum at the N3 treatment. The order of per-plant RLD among treatments was N3 > N2 > N4 > N1 > N5 > N0. In contrast to D6, the D10.5 treatment significantly reduced per-plant RLD under all N levels ($p < 0.05$). However, at the population level (Figure 8A,B), population RLD under D10.5 was significantly higher than that under D6 at the corresponding N levels, with relative increases of 59.5%, 48.7%, 54.4%, 66.7%, 38.7% and 62.5%, respectively ($p < 0.05$). The strongest population compensatory effect was observed with the N3 treatment, followed by N2. Increasing planting density significantly promoted the growth of the population root system; furthermore, both planting density and N application rate exerted a statistically significant effect on the RLD of cotton (Table 3).

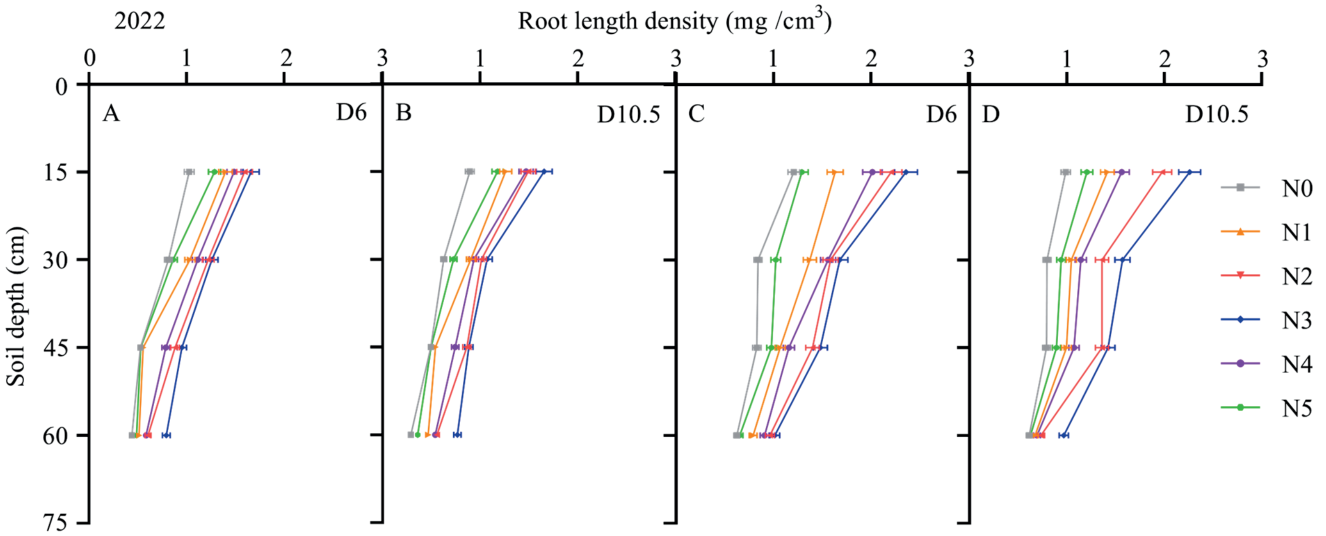

Figure 6. Root length density of cotton with different densities and N application rates (2022). Figures (**A**,**B**) show the flowering and bolling stage, and Figures (**C**,**D**) show the boll opening stage. Error bars represent the standard error (SE).

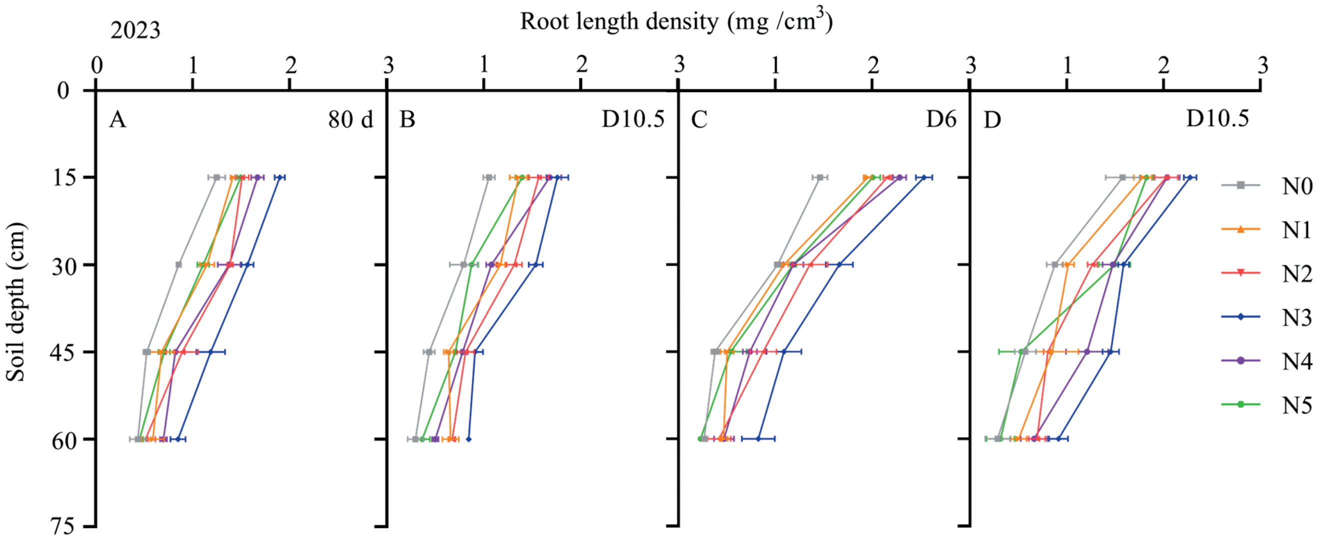

Figure 7. Root length density of cotton with different densities and N application rates (2023). Figures (**A**,**B**) show the flowering and bolling stage, and Figures (**C**,**D**) show the boll opening stage. Error bars represent the standard error (SE).

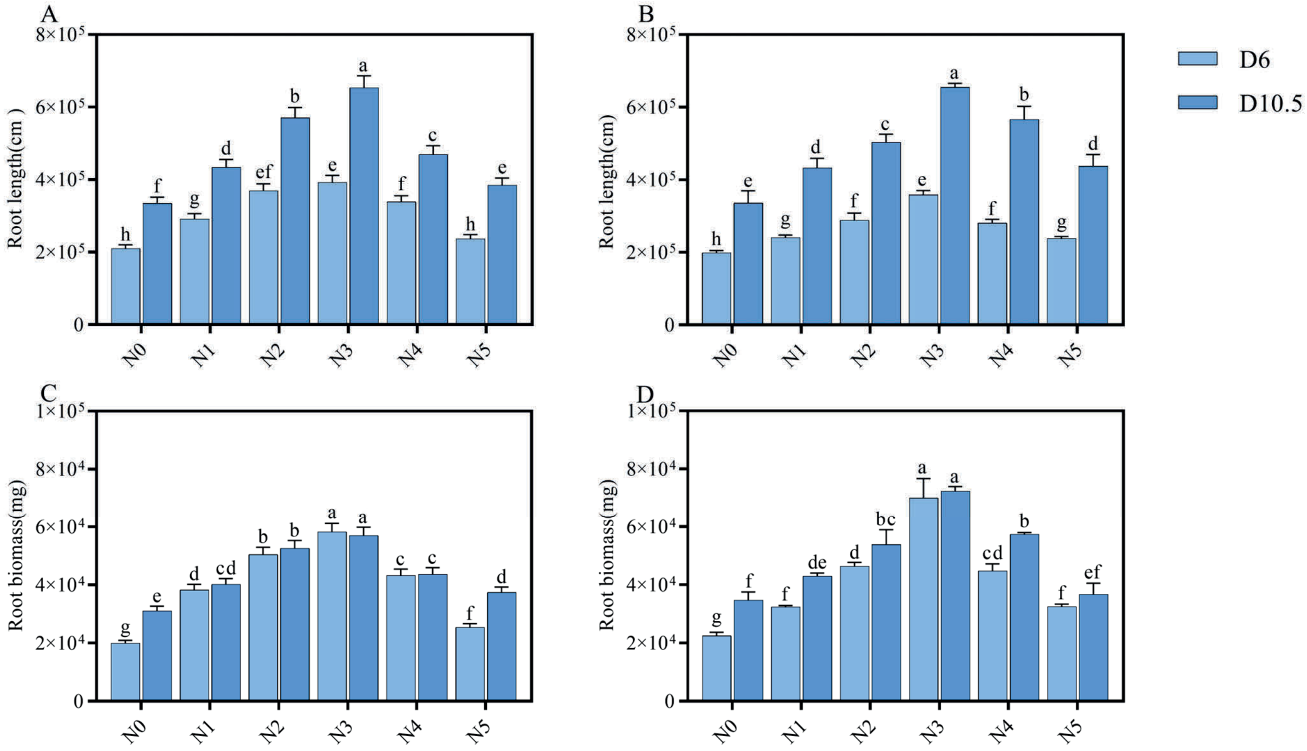

Figure 8. Effects of density and N application rate on root length density and root biomass density of cotton population (boll opening stage). Figures (**A**,**C**) represent 2022, and Figures (**B**,**D**) represent 2023. Different letters indicate significant differences among treatments at $p < 0.05$. Error bars represent the standard error (SE).

Table 3. Source of variation.

Treatment	Year	Growth Stages	Density (D)	Nitrogen (N)	DxN
PH	2022	FBS	**	**	ns
		BOS	ns	**	*
	2023	FBS	**	**	ns
		BOS	**	**	ns
SD	2022	FBS	**	**	*
		BOS	**	**	*
	2023	FBS	**	**	ns
		BOS	**	**	ns
PARIn	2022	SS	**	**	ns
		EFS	**	**	ns
		FBS	ns	**	ns
		BOS	**	**	ns
RS	2022	BOS-ALRA	*	ns	ns
		BOS-NLR	**	**	ns
PRL	2022	BOS	**	**	**
	2023		**	**	**
PRB	2022		**	**	**
	2023		**	**	*

Table 3. *Cont.*

Treatment	Year	Growth Stages	Density (D)	Nitrogen (N)	DxN
Biomass	2022	BOS-VO	**	**	**
		BOS-RO	**	**	ns
	2023	BOS-VO	**	**	**
		BOS-RO	**	**	**
R/S	2022	FBS	**	**	**

Plant height: PH, Stem diameter: SD, PARIn: photosynthetically active radiation interception rate, RS: Root structure, PRL: population root length, PRB: population root biomass, R/S: root–shoot ratio, FBS: flowering and bolling stage, SS: squaring stage, EFS: early flowering stage, BOS: boll opening stage, ALRA: average lateral root angle, NLR: number of lateral roots, VO: vegetative organ, RO: reproductive organ. * Significance is indicated at the 95% confidence level. ** Extreme significance at the 99% confidence level. "ns" indicates no significance at the 95% confidence level.

Cotton RBD decreased with increasing soil depth, with 85.5%–92.7% of the total root biomass concentrated in the 0–45 cm soil layer (Figures 9 and 10). Under the same N level, D10.5 significantly reduced per-plant RBD compared to D6 ($p < 0.05$). However, at the population level, D10.5 exerted a significant promoting effect on RBD ($p < 0.05$). In the first-year experiment, increasing planting density resulted in no significant difference in population RBD among the N1–N4 treatments (Figure 8C,D). However, the RBD values of the D10.5N0 and D10.5N5 treatments were significantly higher than those of the D6N0 and D6N5 treatments ($p < 0.05$). In the 2023 experiment, there was no significant difference in RBD between the two densities under the N3 and N5 treatments. Under the N0, N1, N2 and N4 treatments, the population RBD at the D10.5 density was significantly higher than that at the D6 density ($p < 0.05$), with increases of 54.0% (N0), 32.8% (N1), 16.2% (N2) and 28.3% (N4), respectively. Furthermore, the interaction effect between planting density and N application rate on cotton RBD was significant (Table 3).

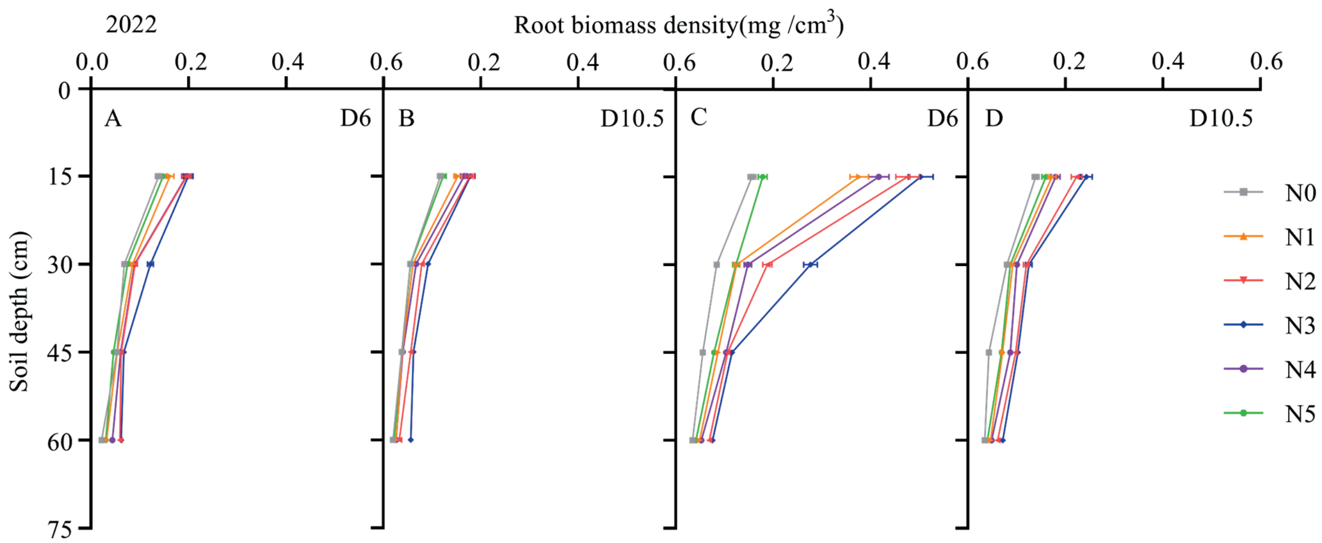

Figure 9. Cotton root biomass density at different densities and N levels (2022). Figures (**A**,**B**) represent the flowering and bolling stage, and Figures (**C**,**D**) represent the boll opening stage. Error bars represent the standard error (SE).

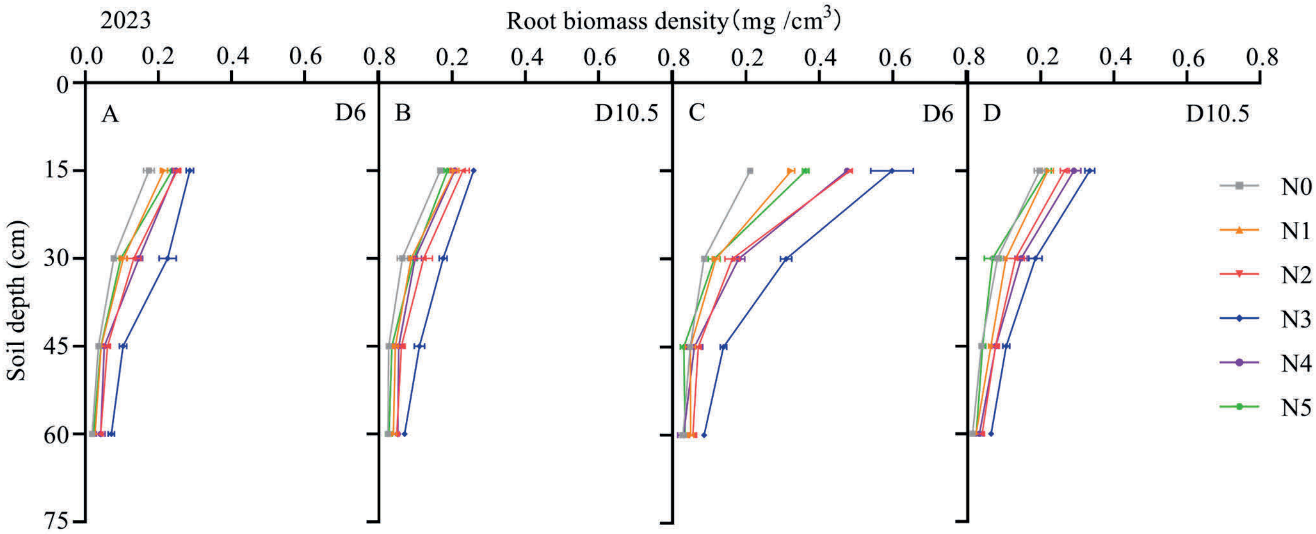

Figure 10. Cotton root biomass density at different densities and N levels (2023). Figures (**A**,**B**) show the flowering and bolling stage, and Figures (**C**,**D**) show the boll opening stage. Error bars represent the standard error (SE).

3.3. Effects on the Accumulation and Distribution of Dry Matter

Both planting density and N application rate had significant effects on the dry matter partitioning of cotton (Figure. 11). N application significantly increased dry matter accumulation in both VODM and RODM of cotton, and this accumulation showed a trend of first increasing and then stabilizing with the increase in N application rate. The maximum dry matter accumulation was achieved under the N3 treatment, with no significant differences observed compared with the N4 and N5 treatments. Under the same N application rate, increasing the planting density significantly enhanced RODM in cotton ($p < 0.05$) (Figure 11B,D). In the 2022 experiment, under high-density conditions, no significant differences in RODM accumulation were detected, starting from the N2; the increases in RODM accumulation under the D10.5 treatment relative to the D6 were 10.1% (N0), 13.5% (N1), 10.8% (N2), 11.5% (N3), 11.9% (N4) and 11.7% (N5). A similar trend was observed in the 2023 experiment. Furthermore, results from both experimental years indicated that the D10.5×N5 treatment had the lowest proportion of RODM. In conclusion, compared with the D6 treatment, the D10.5 significantly increased the RODM of cotton.

3.4. Influence on the Root–Shoot Ratio

With increasing N application rate, the cotton root–shoot ratio (R/S) exhibited a trend of first remaining stable and then decreasing (Figure 12). Under D6, the R/S ratio was the highest in the N0 treatment. Specifically, increasing planting density (i.e., D10.5) significantly increased the R/S ratio under all N application rates ($p < 0.05$). Compared to D6, the relative increases in R/S ratio of D10.5 under N0–N5 were 33.2% (N0), 48.4% (N1), 61.6% (N2), 32.4% (N3), 28.5% (N4) and 23.0% (N5), respectively. Among these, no significant difference was observed in the R/S ratio of D10.5 among the N0–N3 treatments; however, compared to N3, the R/S ratio of D10.5 under N4 and N5 decreased significantly ($p < 0.05$) by 8.5% (N4) and 14.9% (N5), respectively. Furthermore, the interactive effect of planting density and N application on the R/S of cotton was statistically significant (Table 3).

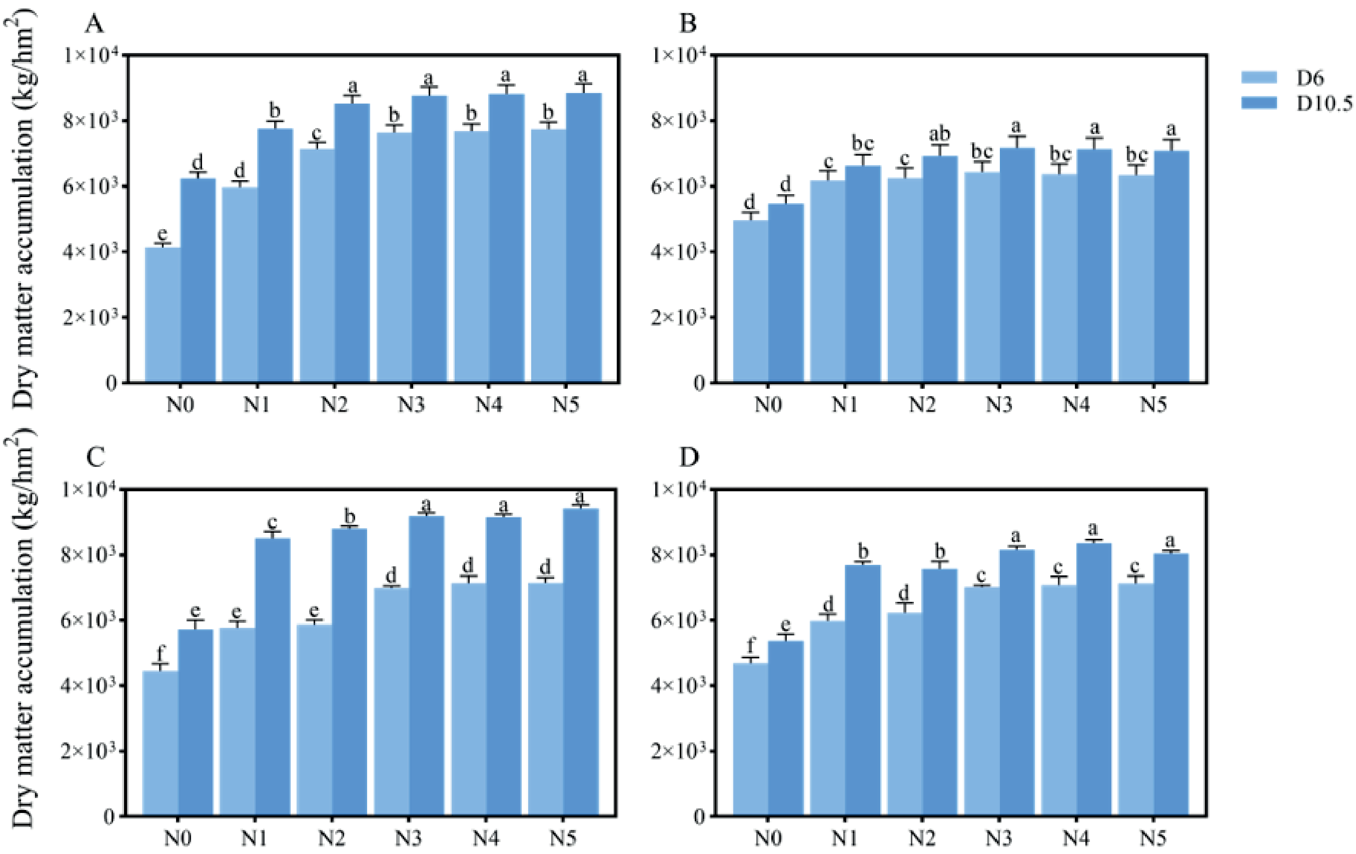

Figure 11. Effects of density and N application rate on dry matter accumulation and distribution in cotton (boll opening stage). (**A**) represents the vegetative organs in 2022, (**B**) represents the reproductive organs in 2022, (**C**) represents the vegetative organs in 2023 and (**D**) represents the reproductive organs in 2023. Different letters indicate significant differences among treatments at $p < 0.05$. Error bars represent the standard error (SE).

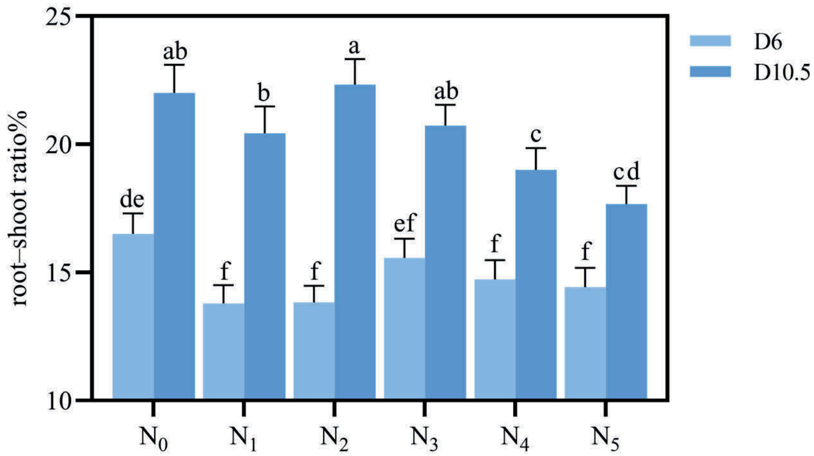

Figure 12. Effects of plant density and N application rate on the root–shoot ratio of cotton at the flowering and bolling stage (2022). Different letters indicate significant differences among treatments at $p < 0.05$. Error bars represent the standard error (SE).

3.5. Impact on Cotton Yield

Planting density and N application rate exerted significant effects on cotton bolls per unit area and SCY, while their effects on boll weight were minimal and there was no significant difference in lint percentage among treatments (Tables 4 and 5). With increasing N application rate, both bolls per unit area and SCY first increased and then stabilized, reaching the peak under the N3 treatment—with no significant difference from the N4 and N5 treatments. However, in the second experimental year (2023), the D10.5N5 treatment had significantly lower bolls per unit area and SCY than the D10.5N4 and D10.5N3 treatments ($p < 0.05$). Increasing planting density significantly increased population bolls per unit area and SCY ($p < 0.05$). The relative increases in bolls per unit area under D10.5 (vs. D6) were 15.4% (N0), 10.5% (N1), 7.4% (N2), 10.4% (N3), 11.0% (N4) and 10.7% (N5), respectively; the relative increases in SCY under D10.5 (vs. D6) were 1.5% (N0), 3.1% (N1), 2.7% (N2), 4.0% (N3), 1.7% (N4) and 1.8% (N5), respectively—with the largest increases observed in the N3 treatment. Specifically, when the treatment combination was D10.5N2, there was no significant difference in bolls per unit area and SCY compared to the D6N3 treatment. This indicates that increasing density with reduced N application can maintain the conventional yield level. When the combination was D10.5N3, both bolls per unit area and SCY reached their maximum values; further increasing N application rate led to stable yield with no additional gains. Collectively, these results confirm that planting density and N application rate have significant effects on cotton bolls per unit area and SCY.

Table 4. Cotton yield and composition with different densities and N application rates (2022).

Treatment	Boll Density ($\times 10^4$/ ha^{-1})	Boll Weight (g)	Lint Percentage (%)	Seed Cotton Yield (kg/ha^{-1})
D6 × N0	80.78 ± 2.04 f	4.45 ± 0.25 ab	38.27 ± 1.03 a	3597.4 ± 90.8 g
D6 × N1	87.18 ± 1.77 e	4.67 ± 0.26 a	38.40 ± 1.00 a	4068.4 ± 82.5 f
D6 × N2	88.54 ± 1.82 de	4.71 ± 0.30 a	38.37 ± 0.49 a	4176.1 ± 86.1 ef
D6 × N3	91.25 ± 0.87 cd	4.75 ± 0.24 a	38.20 ± 0.66 a	4334.4 ± 41.2 bcd
D6 × N4	89.95 ± 0.50 cde	4.85 ± 0.33 a	38.23 ± 0.35 a	4365.6 ± 24.2 bc
D6 × N5	90.40 ± 0.87 cde	4.85 ± 0.35 a	38.40 ± 0.40 a	4381.4 ± 10.0 bc
D10.5 × N0	93.19 ± 3.71 bc	3.92 ± 0.12 b	38.07 ± 1.16 a	3652.8 ± 145.5 g
D10.5 × N1	96.31 ± 0.92 b	4.35 ± 0.26 ab	38.47 ± 0.57 a	4192.7 ± 40.1 def
D10.5 × N2	95.07 ± 0.75 b	4.51 ± 0.41 a	38.27 ± 0.51 a	4290.8 ± 34.0 cde
D10.5 × N3	100.77 ± 1.77 a	4.47 ± 0.36 a	38.33 ± 0.59 a	4507.8 ± 79.1 a
D10.5 × N4	99.81 ± 2.00 a	4.45 ± 0.21 ab	38.23 ± 0.45 a	4438.2 ± 88.5 a
D10.5 × N5	100.06 ± 3.46 a	4.46 ± 0.25 ab	38.37 ± 0.51 a	4459.0 ± 154.7 a
		Source of variation		
Density (D)	**	*	ns	**
Nitrogen (N)	**	ns	ns	**
D × N	ns	ns	ns	ns

The letters in the table indicate statistically significant differences between treatments ($p \leq 0.05$). Values are expressed as mean ± standard error ($n = 3$). * Significance is indicated at the 95% confidence level. ** Extreme significance at the 99% confidence level. "ns" indicates no significance at the 95% confidence level.

Table 5. Cotton yield and composition with different densities and N application rates (2023).

Treatment	Boll Density ($\times 10^4$/ ha^{-1})	Boll Weight (g)	Lint Percentage (%)	Seed Cotton Yield (kg/ha^{-1})
D6 × N0	76.25 ± 1.83 g	4.45 ± 0.27 ab	38.30 ± 0.56 a	3393.2 ± 99.6 f
D6 × N1	82.93 ± 1.24 f	4.65 ± 0.34 ab	38.30 ± 0.55 a	3859.6 ± 209.2 e
D6 × N2	84.44 ± 0.99 ef	4.73 ± 0.61 ab	38.57 ± 0.45 a	3989.6 ± 153.3 de
D6 × N3	91.95 ± 2.59 cd	4.85 ± 0.72 ab	38.53 ± 0.55 a	4455.7 ± 222.2 bc
D6 × N4	89.80 ± 3.90 de	4.87 ± 0.45 a	38.57 ± 0.49 a	4370.2 ± 85.1 bc
D6 × N5	90.03 ± 3.13 de	4.89 ± 0.68 a	38.43 ± 0.51 a	4400.5 ± 76.1 bc
D10.5 × N0	84.64 ± 6.36 ef	4.03 ± 0.06 b	38.37 ± 0.57 a	3405.7 ± 101.0 f
D10.5 × N1	94.16 ± 3.22 cd	4.43 ± 0.21 ab	38.57 ± 0.59 a	4170.1 ± 109.5 cd
D10.5 × N2	97.07 ± 2.07 bc	4.54 ± 0.28 ab	38.47 ± 0.55 a	4407.8 ± 154.0 bc

Table 5. *Cont.*

Treatment	Boll Density ($\times 10^4$/ ha^{-1})	Boll Weight (g)	Lint Percentage (%)	Seed Cotton Yield (kg/ha^{-1})
D10.5 × N3	104.59 ± 2.90 a	4.58 ± 0.33 ab	38.43 ± 0.56 a	4790.4 ± 166.5 a
D10.5 × N4	101.20 ± 4.12 ab	4.61 ± 0.35 ab	38.47 ± 0.59 a	4663.9 ± 163.3 a
D10.5 × N5	96.48 ± 2.50 bc	4.62 ± 0.29 ab	38.47 ± 0.50 a	4450.5 ± 131.6 bc
		Source of variation		
Density (D)	**	**	ns	**
Nitrogen (N)	**	ns	ns	**
D × N	ns	ns	ns	ns

The letters in the table indicate statistically significant differences between treatments ($p \leq 0.05$). Values are expressed as mean ± standard error (n = 3). ** Extreme significance at the 99% confidence level. "ns" indicates no significance at the 95% confidence level.

3.6. The Influence on the Total Nitrogen Content of the Soil

The majority of N was distributed in the 0–40 cm soil layer (Figure 13). Increasing planting density significantly reduced the total nitrogen (TN) content in the 20–60 cm soil layer ($p < 0.05$). During the flowering and bolling stage, only under the N0 treatment did the TN content in the 0–40 cm soil layer decrease significantly with increasing planting density ($p < 0.05$); no significant difference in TN content was observed between density treatments under other N-applied treatments (N1–N5). In the boll opening stage, the TN content in all soil layers decreased. After increasing planting density, there was no significant decrease in TN content in the 0–20 cm soil layer. In the 20–40 cm soil layer, the TN content under the N3 treatment and N4 treatment decreased significantly ($p < 0.05$), by 16.1% and 8%, respectively. In the 40–60 cm soil layer, the TN content under the N1–N5 treatments decreased significantly ($p < 0.05$), with reductions of 20.5% (N1), 17.4% (N2), 19.5% (N3), 21.5% (N4) and 14.5% (N5), respectively. Collectively, these results indicate that planting density exerts a significant effect on the TN content in the deep soil layer (20–60 cm).

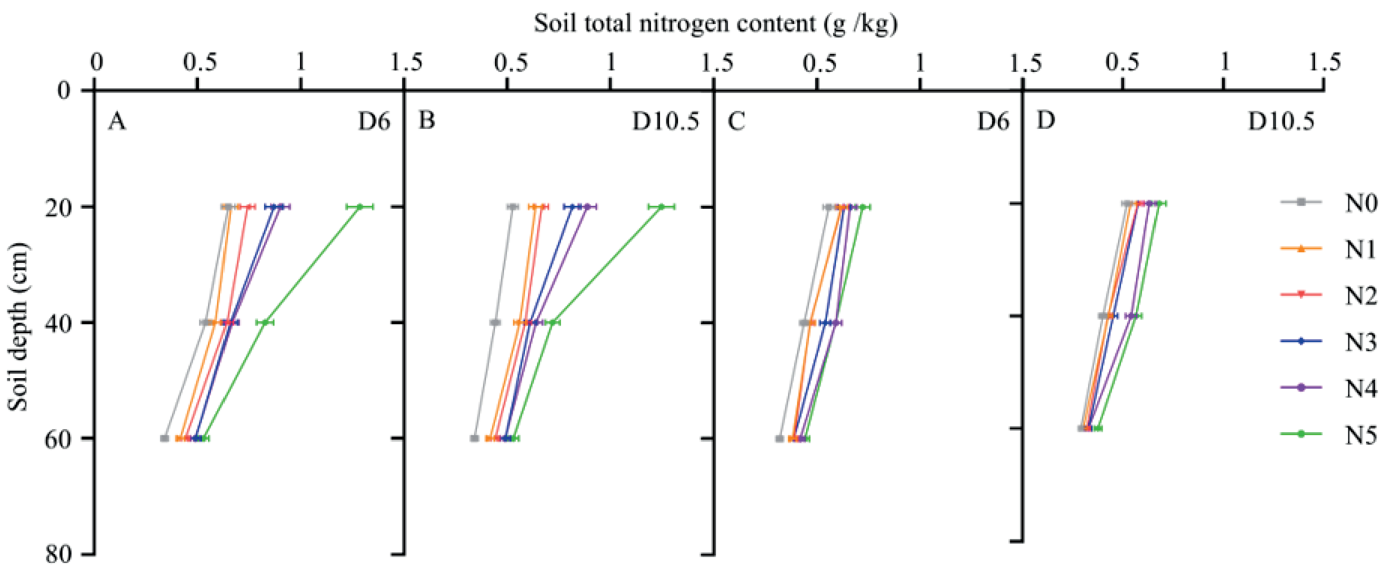

Figure 13. Total nitrogen content of each soil layer depth in cotton fields at different densities and N levels (2022). Figures (**A**,**B**) show N content of each soil layer during the flowering and bolling stage, and Figures (**C**,**D**) show nitrogen content of each soil layer during the flocculation stage. Error bars represent the standard error (SE).

3.7. Effects on the Nitrogen Fertilizer Utilization Efficiency of Cotton

Under the same planting density, with increasing N application rate, NRE, ANUE and PFPN exhibited a significant decreasing trend ($p < 0.05$) (Figure 14 and Table 6). An increase in planting density significantly ($p \leq 0.05$) improved NRE at the nitrogen application levels of N2, N3 and N4, with respective increases of 14.0% (N2), 17.3% (N3) and 17.0% (N4) (Figure 14). Increasing planting density (from D6 to D10.5) significantly increased ANUE ($p < 0.05$). Compared to D6, the relative increases in ANUE of D10.5 under N1–N5 were 14.6% (N1), 10.2% (N2), 16.0% (N3), 2.0% (N4) and 2.2% (N5), respectively—a trend consistent in the second experimental year (2023). At the same N application rate, increasing planting density also increased PFPN; however, significant increases were only observed in specific treatments. In the first year (2022), PFPN of D10.5N1 was significantly higher than that of D6N1, with a relative increase of 3.1% ($p < 0.05$). In the second year (2023), compared with the PFPN under different N application rates at the D6 density, the PFPN of the N1 and N2 treatments at the D10.5 density significantly increased ($p < 0.05$), with increments of 8.0% (N1) and 10.5% (N2), respectively. Additionally, the interaction between planting density and N application rate had a significant effect on cotton ANUE.

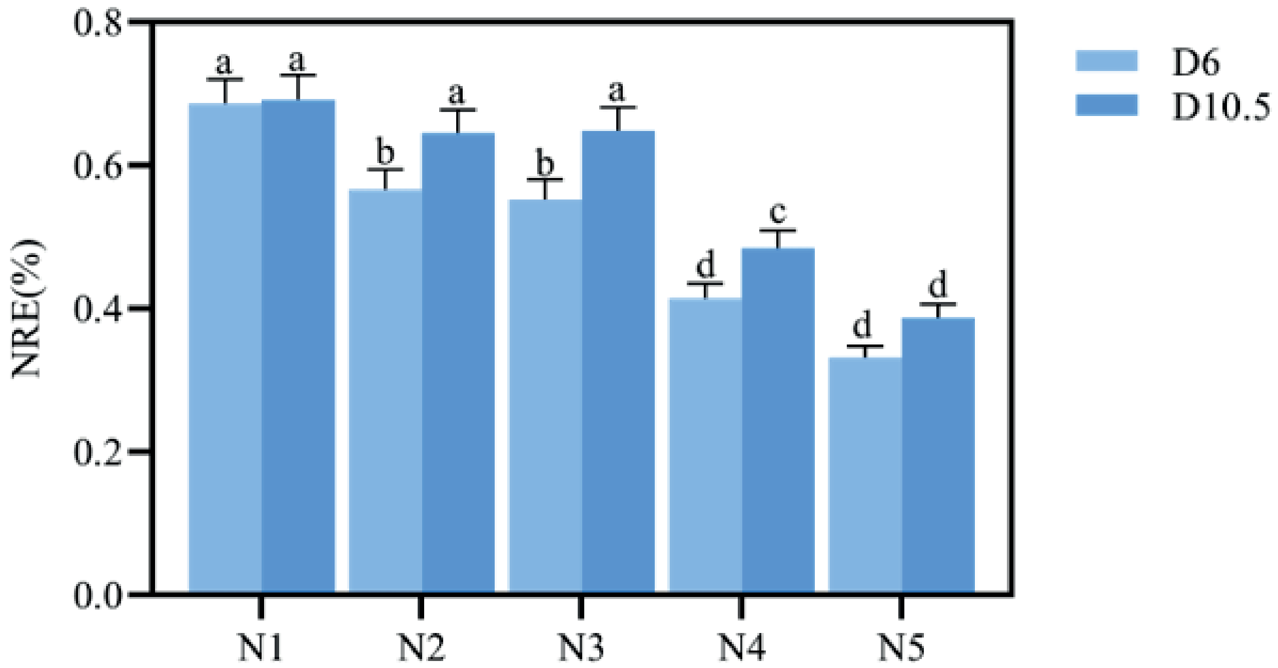

Figure 14. Effects of planting density and N application rate on cotton NRE (2022). Different letters indicate significant differences among treatments at $p < 0.05$. Error bars represent the standard error (SE).

Table 6. NUE of cotton with different densities and N application rates.

Treatment	ANUE (2022) (kg/kg)	ANUE (2023) (kg/kg)	PFPN (2022) (kg/kg)	PFPN (2023) (kg/kg)
D6 × N0				
D6 × N1	6.28 ± 0.31 b	6.22 ± 0.31 bc	54.25 ± 1.10 b	51.46 ± 2.78 b
D6 × N2	3.86 ± 0.19 d	3.98 ± 0.20 e	27.84 ± 0.57 c	26.60 ± 1.02 d
D6 × N3	3.28 ± 0.17 e	4.72 ± 0.24 d	19.26 ± 0.19 d	19.80 ± 0.99 e
D6 × N4	2.56 ± 0.13 f	3.26 ± 0.17 f	14.55 ± 0.08 e	14.57 ± 0.28 f
D6 × N5	2.09 ± 0.11 g	2.69 ± 0.14 g	11.68 ± 0.12 f	11.73 ± 0.20 g
D10.5 × N0				
D10.5 × N1	7.20 ± 0.36 a	10.19 ± 0.51 a	55.90 ± 0.53 a	55.60 ± 1.46 a

Table 6. *Cont.*

Treatment	ANUE (2022) (kg/kg)	ANUE (2023) (kg/kg)	PFPN (2022) (kg/kg)	PFPN (2023) (kg/kg)
D10.5 × N2	4.25 ± 0.21 c	6.68 ± 0.33 b	28.61 ± 0.23 c	29.39 ± 1.02 c
D10.5 × N3	3.80 ± 0.19 d	6.15 ± 0.31 c	20.03 ± 0.35 d	21.29 ± 0.74 e
D10.5 × N4	2.62 ± 0.13 f	4.19 ± 0.21 e	14.79 ± 0.30 e	15.55 ± 0.55 f
D10.5 × N5	2.15 ± 0.11 g	2.79 ± 0.14 fg	11.89 ± 0.42 f	11.87 ± 0.35 g
Source of variation				
Density (D)	**	**	**	**
Nitrogen (N)	**	**	**	**
D × N	**	**	ns	ns

The letters in the table indicate statistically significant differences between treatments ($p \leq 0.05$). Values are expressed as mean ± standard error (n = 3). ** Extreme significance at the 99% confidence level. "ns" indicates no significance at the 95% confidence level.

3.8. Regression Analysis and Correlation Analysis

To further investigate the relationship between N application rate and yield, regression equations between N application rate and SCY were established. As shown in Table 7, increasing planting density reduced the optimal N application rate, with decreases of 9.3% and 21.9% in 2022 and 2023, respectively.

Table 7. Regression analysis of nitrogen application rate and SCY.

Treatment		Regression Equation	R^2	Theoretical Optimal Nitrogen Application Rate (kg/ha^{-1})
2022 year	D6	$y = -0.0082\,x^2 + 4.9671x + 3645.2$	0.9677 *	302.8
	D10.5	$y = -0.0104\,x^2 + 5.7869x + 3706.5$	0.9508 *	274.0
2023 year	D6	$y = -0.0097\,x^2 + 6.3016x + 3394.6$	0.9540 *	324.7
	D10.5	$y = -0.0201\,x^2 + 10.255x + 3430.7$	0.9792 *	253.7

R^2, determination coefficient; * Significant at 95% confidence level.

Figure 15 shows that PH, SD, root length, root biomass, ALRA and NLR all exhibited a significantly positive correlation with SCY. SD, STN content, ALRA and NLR roots showed a negative correlation with the R/S ratio. Additionally, PH and root-related indicators all exhibited a positive correlation with cotton plant dry matter accumulation.

A partial least squares path modeling (PLS-PM) approach was employed to analyze the direct and indirect effects of planting density and N levels on cotton root system characteristics, STN content, VODM, RODM and yield (Figures 16 and 17). Root system characteristics were positively influenced by nitrogen levels (0.659) and planting density (0.102). STN content was positively affected by N levels (0.946) but negatively influenced by planting density (−0.102). There was a negative effect between root system characteristics and STN content (−0.220), and between STN content and RODM (−0.160). RODM exerted a positive effect on yield (0.949). Overall, our data showed a good fit to the hypothetical model, with a goodness-of-fit (GOF) value of 0.690.

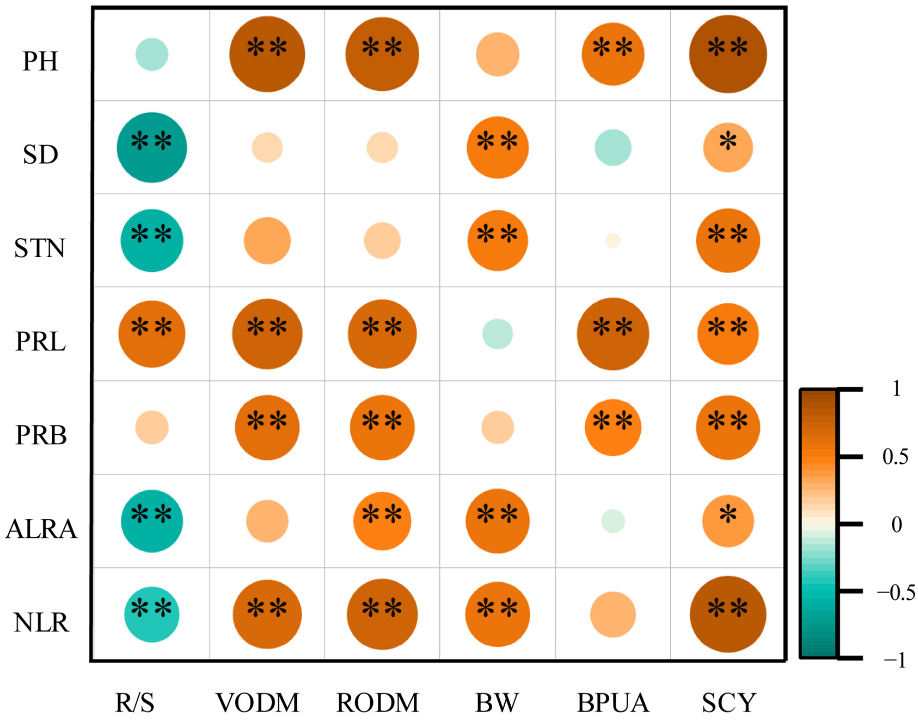

Figure 15. Correlation analysis of root–shoot characteristics and cotton yield under different densities and N application rates. PH: plant height; SD: stem diameter; STN: soil total nitrogen content; PRL: population root length; PRB: population root biomass; ALRA: average lateral root angle; NLR: number of primary lateral roots; R/S: root–shoot ratio; VODM: vegetative organ dry matter; RODM: reproductive organ dry matter; BW: single boll weight; BPUA: number of bolls per unit area; SCY: seed cotton yield. * Significance is indicated at the 95% confidence level. ** Extreme significance at the 99% confidence level.

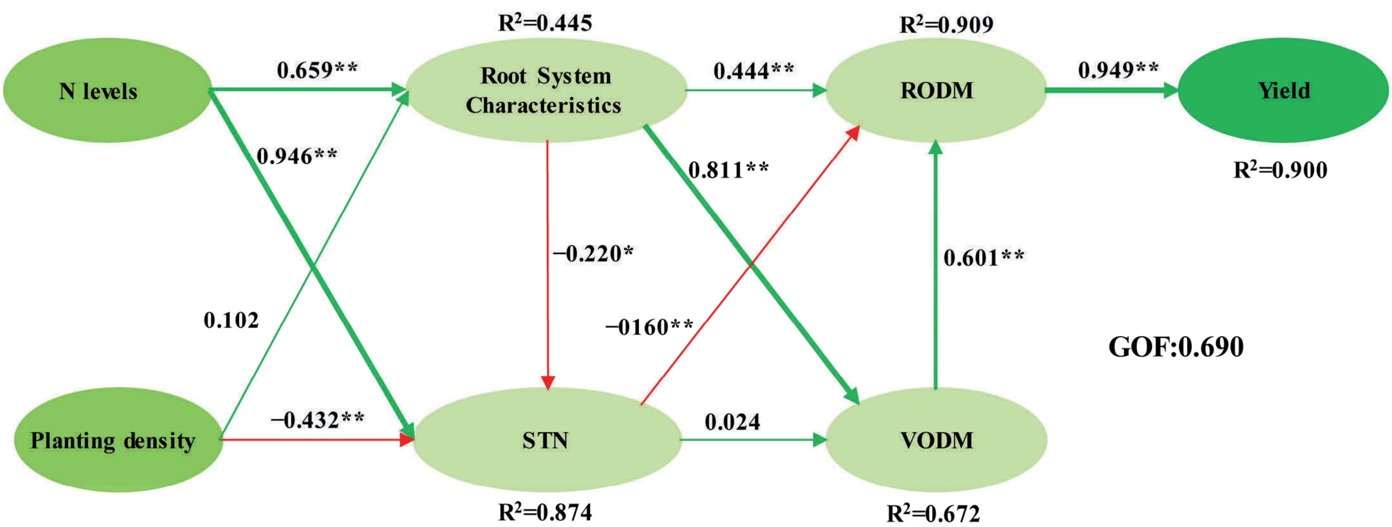

Figure 16. Partial least squares path modeling (PLS-PM) for analyzing direct and indirect effects of planting density and nitrogen levels on cotton root traits, soil total nitrogen content, nutrient organ accumulation, reproductive organ accumulation and yield. The different colored regions represent three parts respectively: treatment, indicator, and yield. Green lines and red lines represent positive effects and negative effects, respectively. The line width is proportional to the strength of factor loadings. * $p < 0.05$ (significant, corresponding to 95% confidence level). ** $p < 0.01$ (highly significant, corresponding to 99% confidence level). R^2 denotes the coefficient of determination for each variable in the inner model.

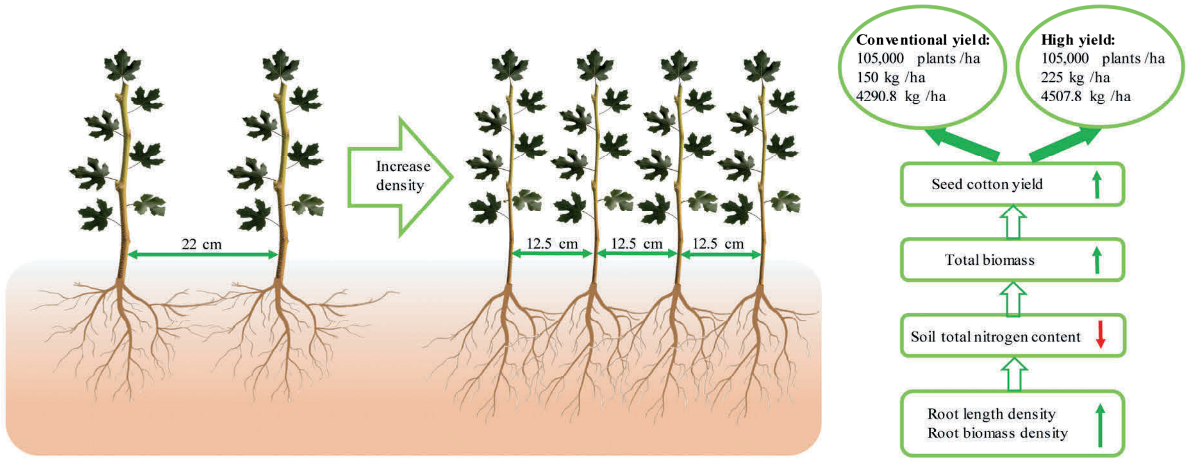

Figure 17. Flowchart illustrating the regulatory effects of increased planting density on cotton growth. In the right section, the solid green arrows indicate a positive correlation, while the solid red arrows indicate a negative correlation. All other arrows only represent direction.

4. Discussion

4.1. Increase Density to Optimize the Root Cap Characteristics of Cotton

Planting density and N application rate exert significant effects on plant root–shoot characteristics. High planting density intensifies inter-plant competition, restricts lateral growth, and promotes upward growth of cotton plants, which is characterized by increased PH and decreased SD [4,23]. Under low planting density, each cotton plant can obtain more nutrients and growth space; in contrast, increasing planting density enables the population to acquire more nutrients, thereby increasing population bolls per unit area and yield. A decrease in PARIn leads to a reduction in dry matter accumulation [33]. This study found that planting density and N application rate had significant effects on the PARIn of the cotton population at the squaring stage. Both increasing planting density and N application rate significantly enhanced the population PARIn, and this trend was consistent with the variation trend of dry matter accumulation, with the maximum value reached at an N application rate of 225 kg N ha^{-1}.

The root system of cotton is the basis for nutrient and water absorption, and the distribution of roots in soil directly affects crop growth and development. The results for ALRA and NLR measured in 2022 indicated that N application could promote the growth and development of lateral roots, while increasing planting density had a promoting effect but it was not significant. Due to the heavy workload, other indicators were focused on and supplemented in the second year to verify the effects of N application rate and planting density on root systems. RLD and RBD can reflect the nutrient and water absorption capacity as well as the spatial expansion ability of the root system [34,35]. Under N deficiency, roots tend to grow vertically; in contrast, sufficient N supply promotes lateral root development [17,36]. Another study found that RLD first increased and then decreased with increasing N application rate, reaching the maximum when the N application rate was 240 kg N ha^{-1} [37]. Under high planting density, the shading effect among plants becomes significant. When plants perceive signals from neighboring plants, they prioritize the allocation of limited photosynthates to the aboveground parts to accelerate vertical growth and compete for sunlight above the canopy, which inevitably leads to a reduction in investment in the root system [38,39]. This is a survival strategy where "expanding sources" (competing for light) takes priority over "reducing consumption" (efficiently absorbing water and nutrients).

For example, in the inland cotton-growing regions of Northwest China, when planting density increased from 135,000 to 180,000 plants ha^{-1}, per-plant root biomass density (RBD) and root length density (RLD) decreased. When planting density increased from 67,500 to 82,500 plants ha^{-1}, population RLD increased significantly with increasing density [40,41]. In this study, a similar pattern was verified in the cotton-growing regions of the Yellow River Basin. For example the NLR, RLD and RBD all exhibited a trend of first increasing and then decreasing with increasing N application rate, reaching their peaks at an N application rate of 225 kg N ha^{-1}. Meanwhile, this N application rate also increased population PARIn and dry matter accumulation. When cotton planting density increased from 60,000 to 105,000 plants ha^{-1}, per-plant RLD and RBD decreased significantly, whereas population RLD and RBD increased significantly. At the same time, this increase in density intensified inter-plant competition and promoted the upward growth of cotton plants.

Excessive N application disrupts the hormonal balance in crops, stimulating the overgrowth of vegetative organs such as stems and leaves. Such excessive vegetative growth leads to crop canopy closure, resulting in poor ventilation and light transmission in the field. Lower leaves exhibit a significant decline in photosynthetic efficiency due to insufficient light, and photosynthates that could be allocated to reproductive growth are consumed unproductively. Meanwhile, as N is no longer scarce for roots, the driving force from nitrogen deficiency stress is lost, leading to a significant weakening of root expansion and growth. Additionally, there is a hypothesis that high-concentration nitrogen fertilizers (especially ammonium nitrogen) can directly impose salt stress on roots, affecting their absorption of other nutrients (e.g., potassium, calcium) and thereby inhibiting root growth [42,43].

4.2. Increasing Density Promotes Coordinated Growth and Enhances Cotton Yield

Dry matter accumulation is a crucial basis for cotton yield formation. Maintaining population dry matter within an appropriate range helps coordinate vegetative and reproductive growth, and construct a reasonable population structure [4]. The two-year experiments in this study showed that increasing planting density significantly increased dry matter accumulation in reproductive organs at population levels; the root–shoot ratio (R/S ratio) began to decrease when the N application rate increased to 225 kg N ha^{-1}. Excessive N application leads to carbon–nitrogen metabolism imbalance, consuming large amounts of carbohydrates and shifting the priority of carbon source allocation to above-ground vegetative organs [44]. This reduces the carbon source allocated to reproductive organs (cotton bolls) and roots, resulting in a decrease in the R/S ratio.

Dry matter accumulation tended to stabilize when the N application rate increased to 150 kg N ha^{-1}, and reached the maximum at 375 kg N ha^{-1}; however, the proportion of dry matter allocated to reproductive organs was the lowest, with values of 44.12% and 46.0% in the two years, respectively. This indicates that excessively high N application results in excessive vegetative growth with delayed maturity, which is unfavorable for dry matter accumulation in reproductive organs and yield formation. For cotton in the Yangtze River Basin, the maximum biomass is achieved under the combination of 120,000 plants ha^{-1} with 120 kg N ha^{-1} or 100,000 plants ha^{-1} with 180 kg N ha^{-1} [45]. Similar conclusions have been reached for other crops. For example, when the density of forage maize increased from 70,000 to 110,000 plants ha^{-1}, dry matter accumulation significantly increased [46]. Our previous study showed that under a planting density of 60,000 plants ha^{-1}, dry matter accumulation began to reach the maximum at an N application rate of 225 kg N ha^{-1} [6]. In summary, although suitable density increase ranges and N application rates vary across different regions, the pattern that increasing density promotes biomass accumulation is consistent with the results of this study.

Planting density and N level exert significant effects on cotton yield formation. Previous studies have demonstrated that, compared with no N application, an N rate of 240 kg N·ha^{-1} significantly increases bolls per unit area and lint yield. When planting density increased from 45,000 to 75,000 plants ha^{-1}, bolls per unit area and lint yield increased, while boll weight decreased [21]. When planting density increased from 60,000 to 120,000 plants ha^{-1}, bolls per unit area and SCY increased significantly, with no significant change in lint percentage [47]. Compared with the conventional N application rate and planting density adopted by local farmers, the treatment with a 33.3% reduction in N application and a 28.6% increase in planting density achieved a higher annual average SCY across the two-year experiment [48]. Similar patterns have been reported in other crops; for example, in soybean, an N application rate of 80 kg N·ha^{-1} combined with an increased planting density of 130,000 plants·ha^{-1} increased yield by 38.7–59.4% [49]. The results of this study showed that the D10.5 treatment significantly increased bolls per unit area and SCY, with no obvious change in boll weight. This phenomenon may be attributed to precise water and fertilizer management, as well as favorable environmental conditions (sufficient sunlight, adequate rainfall and suitable temperatures) during the cotton bolling stage (July and August).

In conclusion, in the cotton-growing regions of the Yellow River Basin, an appropriate increase in planting density can improve SCY. Under the combination of 105,000 plants ha^{-1} (planting density) and 150 kg N ha^{-1} (N application rate), the goal of "increasing density with reduced N application" can be achieved, and there is no significant difference in yield compared with the conventional cultivation combination (60,000 plants ha^{-1} + 225 kg N ha^{-1}). The maximum yield is achieved under the combination of 105,000 plants ha^{-1} and 225 kg N ha^{-1}.

4.3. Increasing Density Significantly Improves Nitrogen Fertilizer Utilization Efficiency and Reduces Total Nitrogen Content in Cotton Field Soil

STN content is an important indicator reflecting soil N supply capacity, while NUE serves as a key basis for optimizing planting density and implementing precise N application. The results of STN content in different soil layers measured in 2022 were relatively obvious. N application significantly increased the TN content in all soil layers, while increasing planting density significantly reduced the TN content in deep soil layers (20–60 cm). In the second year of the experiment, other indicators were focused on and supplemented to elaborate on the objectives of this study. Among influencing factors, planting density and N application rate are critical for regulating STN content and NUE in cotton fields. Previous studies have shown that, compared with no N application treatment, an N application rate of 180 kg N·ha^{-1} significantly increased STN accumulation in the 0–20 cm layer, whereas no significant difference was observed in STN content in deep soil layers [50]. Studies by Aula and Shiwakoti et al. also confirmed that N fertilizer application could significantly increase STN content [51,52]. Additionally, for wheat and rice, increasing planting density led to a decreasing trend in STN content across all soil layers [53,54].

This study found that STN content in the 0–20 cm layer gradually increased with increasing N application rate. Differently from previous studies, when the N application rate exceeded 225 kg N ha^{-1}, STN content in the deep 40–60 cm layer also increased significantly—indicating that excessive N application may cause N migration to deep soil layers. Furthermore, increasing planting density significantly reduced STN content in the 20–60 cm layer.

Increasing planting density can improve NUE. Previous studies have shown that when cotton planting density increased from 120,000 to 195,000 plants ha^{-1} with a 30% reduction in N application, ANUE significantly increased [8]. When planting density increased to 75,000 plants ha^{-1} with an N application rate of 112.5 kg N ha^{-1}, ANUE also reached a relatively high level [55]. Similarly, for maize, when planting density increased from 70,000 to 110,000 plants ha^{-1}, N accumulation and PFPN increased by 21.2% and 15.8%, respectively [46]. N level significantly affects cotton NUE. Insufficient soil N supply occurs at N rates that are too low, while excessive soil N supply happens when the N rate reaches 315 kg·ha^{-1}; both scenarios reduce NUE across all planting densities [56]. This study further found that N application significantly increased STN content in the 0–60 cm layer of cotton fields but decreased ANUE and PFPN; in contrast, high planting density reduced STN content while increasing ANUE and PFPN, which helps avoid excessive soil nitrogen accumulation.

A planting density of 105,000 plants per hectare is currently the recommended density for mechanized harvesting in the Yellow River Valley cotton region. In the Xinjiang cotton region, when the planting density exceeds 105,000 plants per hectare, NUE, SCY, VODM and RODM continue to increase [57,58]. Due to the significant climatic differences between the Xinjiang and Yellow River Valley cotton regions, further research is needed to determine whether nitrogen uptake efficiency will continue to improve with further increases in planting density. Based on the reviewed data on cotton seed and nitrogen fertilizer costs, reducing nitrogen fertilizer application by 33% in exchange for a 43% increase in planting density is economically feasible for farmers [59,60]. Since NUE in China is significantly lower than in developed countries [2,3], it is necessary to reduce nitrogen input and improve NUE.

In summary, the reduction in individual plant size accompanied by enhanced population performance under high planting density is a trade-off strategy for plants to cope with competition. Through the formation of a dense population root network, the efficiency of nitrogen capture is improved. An N application rate of 225 kg N ha^{-1} is the optimal point where the inherent soil nitrogen supply of the experimental field matches the nitrogen demand of cotton. Both insufficient and excessive N application will lead to low NUE and imbalance in cotton growth. Given the low inherent soil fertility of the experimental field, it requires a relatively higher amount of additional N fertilizer—this is the reason for the difference in the optimal N application rate compared with other high-yield cotton fields.

Currently, planting density enhancement technology enables the better establishment of a well-uniformed "small individual, large population" crop stand, laying a solid foundation for the implementation of full-process mechanization. "Increasing density while reducing nitrogen input" is also a promising agronomic conceptual model; however, remote sensing, Geographic Information System (GIS), and Artificial Intelligence (AI) technologies can transform it into a digital model characterized by precise quantification, dynamic optimization, and large-scale replicability. The core of future research will focus on accurate data sensing, intelligent model-based decision-making, and in-depth closed-loop validation with agronomic practices. This research will not only provide transformative technical approaches for cotton production but also set a benchmark for smart cultivation of field crops.

5. Conclusions

Two-year field experiments were conducted to investigate the effects of different planting densities and N application rates on cotton yield. The key results are as follows: the combination of 105,000 plants ha^{-1} (D10.5) and 225 kg N ha^{-1} (N3) achieved the highest SCY; no significant difference in yield was observed between the D10.5N2 (105,000 plants ha^{-1} + 150 kg N ha^{-1}) and D6N3 (60,000 plants ha^{-1} + 225 kg N ha^{-1}) combinations. These findings demonstrate that an appropriate increase in planting density can maintain stable cotton yield while N input is reduced. The underlying mechanism is primarily attributed to three synergistic effects under the optimal density (D10.5): significant increases in population RBD and RLD, which enhance root nutrient absorption capacity; an optimized canopy structure that improves PARIn; and increased bolls per unit area. Collectively, these effects promote both SCY and NUE.

However, the results of this study have regional limitations, as they were derived from a single experimental site. In the future, network experiments will be implemented at typical ecological sites (e.g., Shandong and Hebei provinces) within the cotton-growing regions of the Yellow River Basin. These experiments aim to verify and fine-tune the "increasing density with reduced N application" technical model, ultimately developing refined parameterized cultivation schemes tailored to different sub-ecological zones.

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Abbreviations

The following abbreviations are used in this manuscript:

NUE	Nitrogen Use Efficiency
PH	Plant Height
SD	Stem Diameter
PARIn	Photosynthetically Active Radiation Interception rate
NRE	Nitrogen Recovery Efficiency
ANUE	Agricultural Nitrogen Use Efficiency
PFPN	Partial Factor Productivity of Nitrogen
R/S	Root–Shoot ratio
NLR	Number of Lateral Roots
ALRA	Average Lateral Root Angle
STN	Soil Total Nitrogen
VODM	Vegetative Organ Dry Matter
RODM	Reproductive Organ Dry Matter
BPUA	Number of Bolls Per Unit Area
SCY	Seed Cotton Yield
SE	Standard Error
DAS	Days After Sowing

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